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**(54) Title:** CLONING AND SEQUENCING OF ALLERGENS OF DERMATOPHAGOIDES (HOUSE DUST MITE)

**(57) Abstract**

The present invention features isolated DNA encoding allergens of *Dermatophagoides* (house dust mites) particularly of the species *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, which are protein allergens or peptides which include at least one epitope of the protein allergen. In particular, the invention provides DNA encoding the major *D. farinae* allergens, *Der f* I and *Der f* II and DNA encoding the major *D. pteronyssinus* allergens, *Der p* I and *Der p* II. The present invention further relates to proteins and peptides encoded by the isolated *D. farinae* and *D. pteronyssinus* DNA, including proteins containing sequence polymorphisms. In addition, the proteins or peptides encoded by the isolated DNA, their use as diagnostic and therapeutic reagents and methods of diagnosing and treating sensitivity to house dust mite allergens, are disclosed.

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# CLONING AND SEQUENCING OF ALLERGENS OF DERMATOPHAGOIDES (HOUSE DUST MITE)

## Description

### Background

Recent reports have documented the importance of responses to the Group I and Group II allergens in house dust mite allergy. For example, it has been documented that over 60% of patients have at least 50% of their anti-mite antibodies directed towards these proteins (Lind, P. et al., Allergy, 39:259-274 (1984); van der Zee, J.S. et al., J. Allergy Clin. Immunol., 81:884-896 (1988)). It is possible that children show a greater degree of reactivity (Thompson, P.J. et al., Immunology 64:311-314 (1988)). Allergy to mites of the genus Dermatophagoides (D.) is associated with conditions such as asthma, rhinitis and ectopic dermatitis. Two species, D. pteronyssinus and D. farinae, predominate and, as a result, considerable effort has been expended in trying to identify the allergens produced by these two species. D. pteronyssinus mites are the most common Dermatophagoides species in house dust in Western Europe and Australia. The species D. farinae predominates in other countries, such as North America and Japan (Wharton, G.W., J. Medical Entom, 12:577-621 (1976)). It has long been recognized that allergy to mites of this genus is associated with diseases such as asthma, rhinitis and atopic dermatitis. It is still not clear what allergens produced by these mites are responsible for the allergic response and associated conditions.

### Summary of the Invention

The present invention relates to isolated DNA which encodes a protein allergen of Dermatophagoides ((D.) house dust mite) or a peptide which includes at least one epitope of a protein allergen of a house dust mite of the genus Dermatophagoides. It particularly relates to DNA encoding major allergens of the species D. farinae, designated Der f I and Der f II, or portions of these major allergens (i.e., peptides which include at least one epitope of Der f I or of Der f II). It also particularly relates to DNA encoding major allergens of D. pteronyssinus, designated Der p I and Der p II, or portions of these major allergens (i.e., peptides which include at least one epitope of Der p I or of Der p II).

The present invention further relates to proteins and peptides encoded by the isolated Dermatophagoides (e.g., D. farinae, D. pteronyssinus) DNA including proteins containing sequence polymorphisms. Several nucleotide and resulting amino acid sequence polymorphisms have been discovered in the Der p I, Der p II and Der f II allergens. All such nucleotide variations and proteins, or portions thereof, containing a sequence polymorphism are within the scope of the invention.

Peptides of the present invention include at least one epitope of a D. farinae allergen (e.g., at least one epitope of Der f I or Der f II) or at least one epitope of a D. pteronyssinus allergen (e.g., at least one epitope of Der p I or of Der p II). It also relates to antibodies specific for D. farinae proteins or peptides and to antibodies specific for D. pteronyssinus proteins or peptides.

Dermatophagoides DNA, proteins and peptides of the present invention are useful for diagnostic and therapeutic purposes. For example, isolated D. farinae proteins or peptides can be used to detect sensitivity in an individual to house dust mites and can be used to treat sensitivity (reduce sensitivity or desensitize) in an individual, to whom therapeutically effective quantities of the D. farinae protein or peptide is administered. For example, isolated D. farinae protein allergen, such as Der f I or Der f II, can be administered periodically, using standard techniques, to an individual in order to desensitize the individual. Alternatively, a peptide which includes at least one epitope of Der f I or of Der f II can be administered for this purpose. Isolated D. pteronyssinus protein allergen, such as Der p I or Der p II, can be administered as described for Der f I or Der f II. Similarly, a peptide which includes at least one Der p I epitope or at least one Der p II epitope can be administered for this purpose. A combination of these proteins or peptides (e.g., Der f I and Der f II; Der p I and Der p II; or a mixture of both Der f and Der p proteins) can also be administered. The use of such isolated proteins or peptides provides a means of desensitizing individuals to important house dust mite allergens.

## Brief Description of the Drawings

Figures 1A and 1B show the nucleotide and predicted amino acid sequence of cDNA ggt11 p1(13T) (SEQ ID NOS: 1 and 2, respectively). Numbers to the right are nucleotide positions whereas numbers above the sequence are amino acid positions. Positive amino acid residue numbers correspond to the sequence of the mature excreted Der p I beginning with threonine. Negative sequence numbers refer to the proposed transient pre- and preproenzyme forms of Der p I. The arrows indicate the beginning of the proposed proenzyme sequence and the mature Der p I, respectively. Residues -15 to -13 enclosed by an open box make

up the proposed cleavage for the proenzyme formation, and the dashed residues 52-54 represent a potential N-glycosylation site. The termination TAA codon and the adjacent polyadenylation signal are underlined. Amino acid residues 1-41, 79-95, 111-142, and 162-179 correspond to known tryptic peptide sequences determined by conventional amino acid sequencing analysis.

Figure 2 shows the restriction map of the cDNA insert of clone ggt11 p1(13T) and the strategy of DNA sequencing. Arrows indicate directions in which sequences were read.

Figure 3 is a comparison of N-terminal sequences of Der p I and Der f I. The amino acid sequence for Der p I is equivalent to amino acids 1-20 in Figures 1A and 1B; the Der f I sequence is from reference (12).

Figure 4 shows the reactivity of ggt11 p1(13T) with anti-Der p I. Lysates from Y1089 lysogens induced for phage were reacted by dot-blot with rabbit anti-Der p I (Der p I) or normal rabbit serum (Nrs). Dots (2ml) were made in triplicate from lysates of bacteria infected with ggt11 p1(13T) (a) or ggt11 (b). When developed with $^{125}$I-protein A and autoradiography only the reaction between ggt11 p1(13T) lysate and the anti-Der p I showed reactivity.

Figure 5 shows reaction of clone pGEX-p1(13T) with IgE in allergic serum. Overnight cultures of pGEX or pGEX-p1 where diluted 1/10 in broth and grown for 2 hours at 37°C. They were induced with IPTG, grown for 2 hours at 37°C. The bacteria were pelletted and resuspended in PBS to 1/10 the volume of culture media. The bacteria were lysed by freeze/thaw and sonication. A radioimmune dot-blot was performed with 2ml of these lysates using mite-allergic or non-allergic serum. The dots in row 1 were from E. coli containing pGEX and row 2-4 from different cultures of E. coli infected with pGEX-p1(13T). Reactivity to pGEX-p1(13T) was found with IgE in allergic but not non-allergic serum. No reactivity to the vector control or with non-allergic serum was found.

Figure 6 shows seroreactivity of cDNA clones coding for Der p II in plaque radioimmune assay. Segments of nitrocellulose filters from plaque lifts were taken from clones 1, 3, A, B and the vector control Ampl. These were reached by immunoassay for human IgE against allergic serum (AM) in row 1, non-allergic serum (WT) in row 2 and by protein A immunoassay for Der p I with rabbit antiserum in row 3. The clones 1, 3 and B reacted strongly with allergic serum but not non-allergic or vector control. (Clone B and vector control were not tested with non-allergic serum).

Figures 7A and 7B show the nucleotide and predicted amino acid sequence of cDNA of ggt11 p II (C1) (SEQ ID NOS: 3 and 4, respectively). Numbers to the right are nucleotide positions and numbers above are amino acid positions. Positive numbers for amino acids begin at the known N-terminal of Der p II and match the known sequence of the first 40 residues. Residues -1 to -16 resemble a typical leader sequence with a hydrophobic core.

Figure 8 shows the N-terminal amino acid homology of Der p II and Der f II. (Der f II sequence from reference 30).

Figure 9 is a restriction map of the cDNA insert of clone ggt11 f 1, including a schematic representation of the strategy of DNA sequencing. Arrows indicate directions in which sequences were read.

Figures 10A and 10B are the nucleotide sequence and the predicted amino acid sequence of cDNA ggt11 f 1 (SEQ ID NOS: 5 and 6, respectively). Numbers above are nucleotide positions; numbers to the left are amino acid positions. Positive amino acid residue numbers correspond to the sequence of the mature excreted Der f I beginning with threonine. Negative sequence numbers refer to the signal peptide and the proenzyme regions of Der f I. The arrows indicate the beginning of the proenzyme sequence and the mature Der f I, respectively. The underlined residues -81 to -78 make up the proposed cleavage site for the proenzyme formation, while the underlined residues 53-55 represent a potential N-glycosylation site. The termination TGA codon and the adjacent polyadenylation signal are also underlined. Amino acid residues 1-28 correspond to a known tryptic peptide sequence determined by conventional amino acid sequencing analysis.

Figure 11 is a composite alignment of the amino acid sequences of the mature Der p I (SEQ ID NO: 11) and Der f I proteins. The numbering above the sequence refers to Der p I. The asterisk denotes the gap that was introduced for maximal alignment. The symbol (.) is used to indicate that the amino acid residue of Der f I at that position is identical to the corresponding amino acid residue of Der p I. The arrows indicate those residues making up the active site of Der p I and Der f I.

Figures 12A and 12 B are a comparison of the amino acid sequence in the pre- and pro-peptide regions of Der f I with those of rat cathepsin H, rat cathepsin L, papain. aleurain. CP1. CP2, rat cathepsin B, CTLA-2, MCP, Der p I and actinidin. Gaps. denoted by dashes. were added for maximal alignment. Double asterisks denote conserved amino acid residues which are shared by greater than 80% of the proenzymes: single asterisks show residues which are conserved in greater than 55% of the sequences. The symbol (.) is used to denote semiconserved equivalent amino acids which are shared by greater than 90% of the proenzyme regions.

Figures 13A and 13B are a hydrophilicity plot of the Der p I mature protein and a hydrophilicity plot of the Der f I mature protein produced using the Hopp-Woods algorithm computed with the Mac Vector Sequence Analysis Software (IBI, New Haven) using a 6 residue window. Positive values indicate relative hydrophilicity and negative values indicating relative hydrophobicity.

Figure 14 is the nucleotide sequence and the predicted amino acid sequence of Der f II cDNA (SEQ ID NOS: 7 and 8, respectively). Numbers to the right are nucleotide positions and numbers above are amino acid residues. The stop (TAA) signal is underlined. The first 8 nucleotides are from the oligonucleotide primer used to generate the cDNA, based on the Der p II sequence.

Figure 15 is a restriction map of Der f II cDNA, which was generated by computer from the sequence data. A map of Der p II similarly generated is shown for comparison. There are few common restriction enzyme sites conserved. Sites marked with an asterisk were introduced by cloning procedures.

Figures 16A, 16B, and 16C show the alignment of Der f II and Der p II cDNA sequences. Numbers to the right are nucleotide position and numbers above are amino acid residues. The top line gives Der p II nucleotide sequence and the second the Der p II amino acid residues. The next two lines show differences of Der f II to these sequences.

Figures 17A and 17B are hydrophilicity plots of Der f II and Der p II using the Hopp-Woods algorithm computed with the Mac Vector Sequence Analysis Software (IBI, New Haven) using a 6-residue window.

Figure 18 is a composite alignment of the amino acid sequences of five Der p I clones (a)-(e) which illustrates polymorphism in the Der p I protein (SEQ ID NO: 11). The numbering refers to the sequence of the Der p I(a) clone. The symbol (-) is used to indicate that the amino acid residue of a Der p I clone is identical to the corresponding amino acid residue of Der p I(a) at that position. The amino acid sequences of these clones indicate that there may be significant variation in Der p I, with five polymorphic amino acid residues found in the five sequences.

Figure 19 is a composite alignment of the amino acid sequences of three Der p II clones (c), (1) and (2) which illustrates polymorphism in the Der p II protein. The numbering refers to the sequence of the Der p II(c) clone. The symbol (.) is used to indicate that the amino acid residue of a Der p II clone is identical to the correspondng amino acid residue of Der p II (c) at that position.

Figure 20 is a composite alignment of the amino acid sequences of six Der f II clones (i.e., pFL1, pFL2, MT3, MT5, MT18 and MT16) which illustrates polymorphism in the Der f II protein (SEQ ID NO: 13). The numbering

refers to the sequences of the Der f pFL1 clone. The symbol (.) is used to indicate that the amino acid residue of a Der f II clone is identical to the corresponding amino acid residue of Der f II pFL1 at that position.

Figures 21A, 21B, and 21C are the nucleotide and predicted amino acid sequences of cDNA ggt11 p1(13T) (SEQ ID NOS: 9 and 10, respectively), including the full length of the preproenzyme form of Der p I. Negative sequence numbers refer to the proposed pre- and preproenzyme forms of Der p I.

## Detailed Description of the Invention

The present invention relates to a nucleotide sequence coding for an allergen from the house dust mite Dermatophagoides and to the encoded Dermatophagoides protein or peptide which includes at least one epitope of the Dermatophagoides allergen. It particularly relates to a nucleotide sequence capable of expression in an appropriate host of a major allergen of D. farinae, such as Der f I or Der f II, or of a peptide which includes at least one epitope of Der f I or of Der f II. It also particularly relates to a nucleotide sequence capable of expression in an appropriate host of a major allergen of D. pteronyssinus, such as Der p I or Der p II, or of a peptide which includes at least one epitope of Der p I or of Der p II. The Dermatophagoides nucleotide sequence is useful as a probe for identifying additional nucleotide sequences which hybridize to it and encode other mite allergens, particularly D. farinae or D. pteronyssinus allergens. Further, the present invention relates to nucleotide sequences which hybridize to a D. farinae protein-encoding nucleotide sequence or a D. pteronyssinus protein-encoding nucleotide sequence but which encode a protein from another species or type of house dust mite, such as D. microceras (e.g., Der m I and Der m II).

The encoded Dermatophagoides mite allergen or peptide which includes at least one Dermatophagoides (Der f I or Der f II; Der p I or Der p II) epitope can be used for diagnostic purposes (e.g., as an antigen) and for therapeutic purposes (e.g., to desensitize an individual). Alternatively, the encoded house dust mite allergen can be a protein or peptide, such as a D. microceras protein or peptide, which displays the antigenicity of or is cross-reacitve with a Der f or a Der p allergen; generally, these have a high degree of amino acid homology.

Accordingly, the present invention also relates to compositions which include a Dermatophagoides allergen (e.g., Der f I allergen, Der f II allergen; Der p I or Der p II allergen or other D. allergen cross-reactive therewith) or a peptide which includes at least one epitope of a Dermatophagoides allergen (Der f I, Der f II, Der p I, Der p II or other D. allergen cross-reactive therewith) individually or in combination, and which can be used for therapeutic applications

(e.g., desensitization). As is described below, DNA coding for major allergens from house dust mites have been isolated and sequenced. In particular, and as is described in greater detail in the Examples, cDNA clones coding for the Der p I, Der p II, Der f I and Der f II allergens have been isolated and sequenced. The nucleotide sequence of each of these clones has been compared with that of the homologous allergen from the related mite species (i.e., Der p I and Der f I; Der p II and Der f II), as has the predicted amino acid sequence of each.

The following is a description of isolation and sequencing of the two cDNA clones coding for Der f allergens and their comparison with the corresponding D. pteronyssinus allergen and a description of use of the nucleotide sequences and encoded products in a diagnostic or a therapeutic context.

Isolation and Sequence Analysis of Der f I

A cDNA clone coding for Der f I, a major allergen from the house dust mite D. farinae, has been isolated and sequenced. A restriction map of the cDNA insert of the clone is represented in Figure 9, as is the strategy of DNA sequencing. This Der f I cDNA clone contains a 1.1-kb cDNA insert encoding a typical signal peptide, a proenzyme region and the mature Der f I protein. The product is 321 amino acid residues; a putative 18 residue signal peptide, an 80 residue proenzyme (pro-peptide) region, and a 223 residue mature enzyme region. The derived molecular weight is 25,191. The nucleotide sequence and the predicted amino acid sequence of the Der f I cDNA are represented in Figures 10A and 10B. The deduced amino acid sequence shows significant homology to other cysteine proteases in the pro-region, as well as in the mature protein. Sequence alignment of the mature Der f I protein with the homologous allergen Der p I from the related mite D. pteronyssinus (Figure 11) revealed a high degree of homology (81%) between the two proteins, as predicted by previous sequencing at the protein level. In particular, the residues comprising the active site of these enzymes were conserved and a potential N-glycosylation site was present at equivalent positions in both mite allergens.

Conserved cysteine residue pairs (31, 71) and (65, 103), where the numbering refers to Der p I. are apparently involved in disulphide bond formation on the basis of the assumed similarity of the three dimensional structure of Der p I and Der f I to that of papain and actinidin, which also have an additional disulphide bridge. The fifth and final cysteine residue for which there is a homologous cysteine residue in papain and actinidin is the active site cysteine (residue 35 in Der f I). It is not unlikely that the two extra cysteine residues present in Der p I and Der f I may be involved in forming a third disulphide bridge.

The potential N-glycosylation site in Der p I is also present at the equivalent position in Der f I, with conservation of the crucial first and last residues of the tripeptide site. The degree of of Der f I and Der p I has yet to be determined. Carbohydrates, including mannose, galactose, N-acetylglucosamine and N-acetylgalactosamine, have been reported in purified preparations of these mite allergens (Chapman, M.D., J. Immunol., 125:587-592 (1980); Wolden, S. et al., Int. Arch. Allergy Appl. Immunol., 68:144-151 (1982)).

Given the degree of homology over the first thirty N-terminal amino acid residues between mature Der p I and Der m I (70%) and mature Der f I and Der m I (97%) with the Der m I residues determined by conventional amino acid sequencing (Platts-Mills TAE et al., In: Mite Allergy, a World-Wide Problem, 27-29 (1988); Lind, P. and N. Horn, In: Mite Allergy, a World-Wide Problem, 30-34 (1988)), it is probable that the full mature Der m I sequence will confirm an overall 70-80% homology between the Group I mite allergens. Der m I is an allergen from D. microceras. High homology between the proenzyme moieties of Der p I and Der f I (91%) over the residues -23 to -1 and the structural analysis of Der f I suggests that the Group I allergens are likely to have N-terminal extension peptides of the mature protein of homologous structure and, at least for the pro-peptide, composition.

Studies on the fine structure of the design of signal sequences have identified three structurally dissimilar regions so far: a positively charged N-terminal (n) region, a central hydrophobic (h) region and a more polar C-terminal (c) region that seems to define the cleavage site (Von Heijne, G., EMBO J., 3:2315-2323 (1984); Eur. J. Biochem., 133:17-21 (1983); J. Mol. Biol., 184:99-105 (1985)). Analysis of the signal peptide of Der f I revealed that it, too, contained these regions (Figures 12A and 12B). The n-region is extremely variable in length and composition, but its net charge does not vary appreciably with the overall length, and has a mean value of about +1.7. The n-region of the Der f I signal peptide, with a length of two residues, has a net charge of +2 contributed by the initiator methionine (which is unformylated and hence positively charged in eukaryotes) and the adjacent lysine (Lys) residue. The h-region of Der f I is enriched with hydrophobic residues, the characteristic feature of this region, with only one hydrophilic residue serine (Ser) present which can be tolerated. The overall amino acid composition of the Der f I c-region is more polar than that of the h-region as is found in signal sequences with the h/c boundary located between residues -6 and -5, which is its mean position in eukaryotes. Thus, the Der f I pre-peptide sequence appears to fulfill the requirements to which a functional signal sequence must conform.

While the signal sequence of Der f I and other cysteine proteases share structural homology, all being composed of the n,h and c-regions, they are highly variable with respect to overall length and amino acid sequence, as is clear in Figures 12A and 12B. However, significant sequence homology has been shown between the pro-regions of cysteine protease precursors (Ishidoh, K. et al., FEBS Letters, 226:33-37 (1987)). Alignment of the proenzyme regions of Der f I and a number of other cysteine proteases (Figures 12A and 12B) indicated that these proregions share a number of very conserved residues as well as semi-conserved residues which were present in over half of the sequences. This homology was increased if conservative amino acids such as valine (Val), isoleucine (Ile) and leucine (Leu) (small hydrophobic residues) or arginine (Arg) and Lys (positively charged residues) were regarded as identical. The Der f I proregion possessed six out of seven highly conserved amino acids and all the residues at sites of conservative changes. The homology at less conserved sites was lower. Homology in the pro-peptide, in particular the highly conserved residues, may be important when considering the function of the pro-peptide in the processing of these enzymes, since it indicates that these sequences probably have structural and functional similarities.

Highly cross-reactive B cell epitopes on Der f I and Der p I have been demonstrated using antibodies present in mouse, rabbit and human sera (Heymann, P.W. et al., J. Immunol. 137:2841-2847 (1986); Platts-Mills, TAE et al., J. Allergy Clin. Immunol. 78:398-407 (1986)). However, species-specific epitopes have also been defined in these systems. Murine monoclonal antibodies bound predominantly to species-specific determinants (Platts-Mills TAE et al., J. Allergy Clin. Immunol. 139:1479-1484 (1987)). Some 40% of rabbit anti-Der p I reactivity was accounted for by epitopes unique to Der p I (Platts-Mills, TAE et al., J. Allergy Clin. Immunol. 78:398-407 (1986)), and some species-specific binding of antibodies from allergic humans was observed, although the majority bind to cross-reactive epitopes (Platts-Mills TAE et al., J. Immunol. 139:1479-1484 (1987)).

The recombinant DNA strategy of gene fragmentation and expression was used (Greene, W.K. et al., Immunol. (1990)) to define five antigenic regions of recombinant Der p I which contained B cell epitopes recognized by a rabbit anti-Der p I antiserum. Using the technique of immunoabsorption, three of these putative epitopes were shown to be shared with Der f I (located on regions containing amino acid residues 34-47, 60-72 and 166-194) while two appeared to be specific for Der p I (regions 82-99 and 112-140). Differences in the reactivity of these peptides to rabbit anti-D. farinae supported the above division into cross-reactive and species-specific epitopes. The sequence differences shown between

the Der p I and the Der f I proteins are primarily located in the N and C terminal regions, as well as in an extended surface loop (residues 85-136) linking the two domains of the enzyme that includes helix D (residues 127-136), as predicted from the secondary and tertiary structures of papain and actinidin (Baker, E.N. and J. Drenth, In: Biological Macromolecules and Assemblies, Vol. 3, pp. 314-368, John Wiley and Sons, NY (1987)). The surface location of these residues is supported by the hydrophilicity plots of Der p I and Der f I in Figures 13A and 13B, which illustrate the predominantly hydrophilic nature of this region that predicts surface exposure. This region also contains the two species-specific B cell epitopes recognized by the rabbit anti-Der p I serum (see above). Analysis of the sequences in the regions containing the cross-reactive epitopes (located in regions 34-47 and 60-72) are completely conserved between Der p I and Der f I, while the majority of residues in a third cross-reactive epitope-containing region (residues region 166-194) were conserved.

Expression of cDNA encoding Der f I results in production of pre-pro-Der f I protein in E. coli, a recombinant protein of greater solubility, stability and antigenicity than that of recombinant Der p I. Protein encoded by Der f I cDNA has been expressed using a pGEX vector and has been shown by radioimmune assay to react with rabbit anti-D. farinae antibodies. The availability of high yields of soluble Der f I allergen and antigenic derivatives will facilitate the development of diagnostic and therapeutic agents and the mapping of B and T cell antigenic determinants.

With the availability of the complete amino acid sequence of recombinant Der f I, mapping of the epitopes recognized by both the B and T cell compartments of the immune system can be carried out. The use of techniques such as the screening of overlapping synthetic peptides, the use of monoclonal antibodies and gene fragmentation and expression should enable the identification of both the continuous and topographical epitopes of Der f I. It will be particularly useful to determine whether allergenic (IgE-binding) determinants have common features and are intrinsically different from antigenic (IgG-binding) determinants and whether T cells recognize unique epitopes different from those recognized by B cells. Studies to identify the Der f I epitopes reactive with mite allergic human IgE antibodies and the division of these into determinants cross-reactive with Der p I and determinants unique to Der f I can also be carried out. B cell (and T cell) epitopes specific for either species can be used to provide useful diagnostic reagents for determining reactivity to the different mite species, while cross-reacting epitopes are candidates for a common immunotherapeutic agent.

As described in detail in the Examples, a cDNA clone coding for Der p I which contained a 0.8-kb cDNA insert has been isolated. Sequence analysis revealed that the 222 amino acid residue mature recombinant Der p I protein showed significant homology with a group of cysteine proteases, including actinidin, papain, cathepsin H and cathepsin B.

Isolation and Sequence Analysis of Der f II

A cDNA clone coding for Der f II, a major allergen from the house dust mite D. farinae, has been isolated and sequenced, as described in the Examples. The nucleotide sequence and the predicted amino acid sequence of the Der f II cDNA are represented in Figure 14. A restriction map of the cDNA insert of a clone coding for Der f II is represented in Figure 15.

Figures 16A, 16B, and 16C show the alignment of Der f II and Der p II cDNA sequences. The homology of the sequence of Der f II with Der p II (88%) is higher than the 81% homology found with Der p I and Der f I, which is significantly different ($p<0.05$) using the $chi^2$ distribution. The reason for this may simply be that the Group I allergens are larger and each residue may be less critical for the structure and function of the molecule. It is known, for example, that assuming they adopt a similar conformation to other cysteine proteases, many of the amino acid differences in Der p I and Der f I lie in residues linking the two domain structures of the molecules. The 6 cysteine molecules are conserved between the group II allergens, suggesting a similar disulphide bonding, although this may be expected, given the high overall homology. Another indication of the conservation of these proteins is that 34/55 of the nucleotide changes of the coding sequence are in the third base of a codon, which usually does not change the amino acid. Residues that may be of importance in the function of the molecule are Ser 57 where all three bases are changed but the amino acid is conserved. A similar phenomenon exists at residue 88, where a complete codon change has conserved a small aliphatic residue. Again, like Der p II, the Der f II cDNA clone does not have a poly A tail, although the 3' non-coding region is rich in adenosine and has two possible polyadenylation signals ATAA. The nucleotides encoding the first four residues are from the PCR primer which was designed from the known homology of Der p II and Der f II from N-terminal amino acid sequencing. A primer based on the C-terminal sequence can now be used to determine these bases, as well as the signal sequence.

Uses of the subject allergenic proteins/peptides and DNA encoding same

The materials resulting from the work described herein, as well as compositions containing these materials, can be used in methods of diagnosing, treating and preventing allergic responses to mite allergens, particularly to mites of the genus Dermatophagoides, such as D. farinae and D. pteronyssinus. In addition, the cDNA (or the mRNA from which it was transcribed) can be used to identify other similar sequences. This can be carried out, for example, under conditions of low stringency and those sequences having sufficient homology (generally greater than 40%) can be selected for further assessment using the method described herein. Alternatively, high stringency conditions can be used. In this manner, DNA of the present invention can be used to identify sequences coding for mite allergens having amino acid sequences similar to that of Der f I, Der f II, Der p I or Der p II. Thus, the present invention includes not only D. farinae and D. pteronyssinus allergens, but other mite allergens as well (e.g., other mite allergens encoded by DNA which hybridizes to DNA of the present invention).

Proteins or peptides encoded by the cDNA of the present invention can be used, for example, as "purified" allergens. Such purified allergens are useful in the standardization of allergen extracts or preparations which can be used as reagents for the diagnosis and treatment of allergy to house dust mites. Through use of the peptides of the present invention, allergen preparations of consistent, well-defined composition and biological activity can be made and administered for therapeutic purposes (e.g., to modify the allergic response of a house dust mite-sensitive individual). Der f I or Der f II peptides or proteins (or modified versions thereof, such as are described below) may, for example, modify B-cell response to Der f I or Der f II, T-cell response to Der f I and Der f II, or both responses. Similarly, Der p I or Der p II proteins or peptides may be used to modify B-cell and/or T-cell response to Der p I or Der p II. Purified allergens can also be used to study the mechanism of immunotherapy of allergy to house dust mites, particularly to Der f I, Der f II, Der p I and Der p II, and to design modified derivatives or analogues which are more useful in immunotherapy than are the unmodified ("naturally-occurring") peptides.

In those instances in which there are epitopes which are cross-reactive, such as the three epitopes described herein which are shared by Der f I and Der p I, the area(s) of the molecule which contain the cross-reactive epitopes can be used as common immunotherapeutic peptides to be administered in treating allergy to the two (or more) mite species which share the epitope. For example, the cross-reactive epitopes could be used to induce IgG blocking antibody against both allergens (e.g., Der f I and Der p I allergen). A peptide containing a

univalent antibody epitope can be used, rather than the entire molecule, and may prove advantageous because the univalent antibody epitope cannot crosslink mast cells and cause adverse reactions during desensitizing treatments. It is also possible to attach a B cell epitope to a carrier molecule to direct T cell control of allergic responses.

Alternatively, it may be desirable or necessary to have peptides which are specific to a selected Dermatophagoides allergen. As described herein, two epitopes which are apparently Der p I-specific have been identified. A similar approach can be used to identify other species-specific epitopes (e.g., Der p I or II, Der f I or II). The presence in an individual of antibodies to the species-specific epitopes can be used as a quick serological test to determine which mite species is causing the allergic response. This would make it possible to specifically target therapy provided to an individual to the causative species and, thus, enhance the therapeutic effect.

Work by others has shown that high doses of allergens generally produce the best results (i.e., best symptom relief). However, many people are unable to tolerate large doses of allergens because of allergic reactions to the allergens. Modification of naturally-occurring allergens can be designed in such a manner that modified peptides or modified allergens which have the same or enhanced therapeutic properties as the corresponding naturally-occurring allergen but have reduced side effects (especially anaphylactic reactions) can be produced. These can be, for example, a peptide of the present invention (e.g., one having all or a portion of the amino acid sequence of Der f I or Der f II, Der p I or Der p II). Alternatively, a combination of peptides can be administered. A modified peptide or peptide analogue (e.g., a peptide in which the amino acid sequence has been altered to modify immunogenicity and/or reduce allergenicity or to which a component has been added for the same purpose) can be used for desensitization therapy.

Administration of the peptides of the present invention to an individual to be desensitized can be carried out using known techniques. A peptide or combination of different peptides can be administered to an individual in a composition which includes, for example, an appropriate buffer, a carrier and/or an adjuvant. Such compositions will generally be administered by injection, inhalation, transdermal application or rectal administration. Using the information now available, it is possible to design a Der p I, Der p II, Der f I or Der f II peptide which, when administered to a sensitive individual in sufficient quantities, will modify the individual's allergic response to Der p I, Der p II, Der f I and/or Der f II. This can be done, for example, by examining the structures of these allergens, producing peptides to be examined for their ability to influence B-

cell and/or T-cell responses in house dust mite-sensitive individuals and selecting appropriate epitopes recognized by the cells. Synthetic amino acid sequences which mimic those of the epitopes and which are capable of down regulating allergic response to Der p I, Der p II, Der f I or Der f II allergens can be made. Proteins, peptides or antibodies of the present invention can also be used, in known methods, for detecting and diagnosing allergic response to Der f I or Der f II. For example, this can be done by combining blood obtained from an individual to be assessed for sensitivity to one of these allergens with an isolated allergenic peptide of house dust mite, under conditions appropriate for binding of or stimulating components (e.g., antibodies, T cells, B cells) in the blood with the peptide and determining the extent to which such binding occurs. Der f and Der p proteins or peptides can be administered together to treat an individual sensitive to both allergen types.

It is now also possible to design an agent or a drug capable of blocking or inhibiting the ability of Der p I, Der p II, Der f I or Der f II to induce an allergic reaction in house dust mite-sensitive individuals. Such agents could be designed, for example, in such a manner that they would bind to relevant anti-Der p I, anti-Der p II, anti-Der f I or anti-Der f II IgEs, thus preventing IgE-allergen binding and subsequent mast cell degranulation. Alternatively, such agents could bind to cellular components of the immune system, resulting in suppression or desensitization of the allergic response to these allergens. A non-restrictive example of this is the use of appropriate B- and T-cell epitope peptides, or modifications thereof, based on the cDNA/protein structures of the present invention to suppress the allergic response to these allergens. This can be carried out by defining the structures of B- and T-cell epitope peptides which affect B- and T-cell function in in vitro studies with blood cells from house dust mite-sensitive individuals.

The cDNA encoding Der p I, Der p II, Der f I or Der f II or a peptide including at least one epitope thereof can be used to produce additional peptides, using known techniques such as gene cloning. A method of producing a protein or a peptide of the present invention can include, for example, culturing a host cell containing an expression vector which, in turn, contains DNA encoding all or a portion of a selected allergenic protein or peptide (e.g., Der p I, Der p II, Der f I, Der f II or a peptide including at least one epitope). Cells are cultured under conditions appropriate for expression of the DNA insert (production of the encoded protein or peptide). The expressed product is then recovered, using known techniques. Alternatively, the allergen or portion thereof can be synthesized using known mechanical or chemical techniques. As used herein, the

term protein or peptide refers to proteins or peptides made by any of these techniques. The resulting peptide can, in turn, be used as described previously.

DNA to be used in any embodiment of this invention can be cDNA obtained as described herein or, alternatively, can be any oligodeoxynucleotide sequence having all or a portion of the sequence represented in Figures 1A and 1B, 7A and 7B, 10A and 10B, and 14 or their functional equivalent. Such oligodeoxynucleotide sequences can be produced chemically or mechanically, using known techniques. A functional equivalent of an oligonucleotide sequence is one which is capable of hybridizing to a complementary oligonucleotide sequence to which the sequence (or corresponding sequence portions) of Figures 1A and 1B, 7A and 7B, 10A and 10B, and 14 hybridizes and/or which encodes a product (e.g., a polypeptide or peptide) having the same functional characteristics of the product encoded by the sequence (or corresponding sequence portion) represented in these figures. Whether a functional equivalent must meet one or both criteria will depend on its use (e.g., if it is to be used only as an oligoprobe, it need meet only the first criterion and if it is to be used to produce house dust mite allergen, it need only meet the second criterion).

The structural information now available (e.g., DNA, protein/peptide sequences) can also be used to identify or define T cell epitope peptides and/or B cell epitope peptides which are of importance in allergic reactions to house dust mite allergens and to elucidate the mediators or mechanisms (e.g., interleukin-2, interleukin-4, gamma interferon) by which these reactions occur. This knowledge should make it possible to design peptide-based house dust mite therapeutic agents or drugs which can be used to modulate these responses.

The present invention will now be further illustrated by the following Examples, which are not intended to be limiting in any way.

## EXAMPLE 1

### MATERIALS AND METHODS

Cloning and Expression of Der p I cDNA.

Polyadenylated mRNA was isolated from the mite Dermatophagoides pteronyssinus cultured by Commonwealth Serum Laboratories, Parkville, Australia, and cDNA was synthesized by the RNA-ase H method (5) using a kit (Amersham, International, Bucks). After the addition of EcoRI linkers the cDNA was ligated into ggt11 and plated in E. coli Y1090 (r-) (Promega Biotec, Madison, Wisconsin), to produce a library of 5x10$^5$ recombinants. Screening was performed by plaque radioimmune assay (6) using a rabbit anti-Der p I antiserum (7). Reactivity was detected by hydrochloride in 0.1

M sodium acetate buffer pH 5.2 were then added and the mixture was homogenized and spun at 10,000 rpm for 30 min in a Sorval SS34 rotor. The supernatant was collected and layered onto a CsCl pad (5ml of 4.8 M CsCl in 10 mM EDTA) and centrifuged at 37,000 rpm for 16h at 15°C in a SW41 TI rotor (Beckman Instruments, Inc., Fullerton, CA). The DNA band at the interphase was collected and diluted 1:15 in l0mM Tris HCl/1 mM EDTA buffer, pH 8.0. Banding of genomic DNA in CsCl was carried out by the standard method.

Isolation of DNA from ggt11 p1 cDNA Clone.

Phage DNA from ggt11 p1 clone was prepared by a rapid isolation procedure. Clarified phage plate lysate (1 ml) was mixed with 270ml of 25% wt/vol polyethylene glycol (PEG 6000) in 2.5 M NaCl and incubated at room temperature for 15 min. The mixture was then spun for 5 min in a microfuge (Eppendorf, Federal Republic of Germany), and the supernatant was removed. The pellet was dissolved in 100 ml of 10 mM Tris/HCl pH 8.0 containing 1 mM EDTA and 100 mM NaCl. This DNA preparation was extracted 3 times with phenol/chloroform (1:1) and the DNA was precipitated by ethanol.

DNA Hybridization.

Nucleic acid was radiolabelled with $^{32}P$ by nick translation (10). DNA samples were digested with appropriate restriction enzymes using conditions recommended by the supplier. Southern blots were prepared using Zeta-Probe membranes (Bio-Rad Laboratories, Richmond, CA). Prehybridization, hybridization, posthybridization washes were carried out according to the manufacturers recommendations (bulletin 1234, Bio-Rad Laboratories).

Cloning and DNA Sequencing

To clone the 0.8-kb cDNA insert from clone ggt11 p1 into plasmid pUC8, phage DNA was digested with EcoRI restriction enzyme and then ligated to EcoRI-digested pUC8 DNA and used to transform Escherichia coli JM83. The resulting recombinant plasmid was designated as pHDM 1.

To obtain clones for DNA sequence analysis, the cDNA insert was isolated from pHDM 1 and ligated to M13-derived sequencing vectors mp18 and mp19 (16). Transformation was carried out using E. coli JM1O7 and sequencing was performed by the dideoxynucleotide chain termination method (11).

RESULTS

Several phage clones reacted with the rabbit anti Der p I serum and hybridized with all 3 oligonucleotide probes. One of these, ggt11 p1(13T), was examined further. The nucleotide sequence of the cDNA insert from this clone, ggt11 p1, was determined using the sequencing strategy shown in Fig. 2. The complete sequence was shown to be 857 bases long and included a 69-base-long 5' proximal end sequence, a coding region for the entire native Der p I protein of 222 amino acids with a derived molecular weight of 25,371, an 89-base-long 3' noncoding region and a poly (A) tail of 33 residues (Figures 1A and 1B).

The assignment of a threonine residue at position 1 as the $NH_2$-terminal amino acid of Der p I was based on data obtained by $NH_2$-terminal amino acid sequencing of the pure protein isolated from mite excretions (17). The predicted amino acid sequence matched with data obtained by amino acid sequence analysis of the $NH_2$-terminal region as well as with internal sequences derived from analyses of tryptic peptides (Figures 1A and 1B). The complete mature protein is coded by a single open reading frame terminating at the TAA stop codon at nucleotide position 736-738. At present, it is not certain whether the first ATG codon at nucleotide position 16-18 is the translation initiation site, since the immediate flanking sequence of this ATG codon (TTGATGA) showed no homology with the Kozak consenses sequence (ACCATGG) for the eukaryotic translation initiation sites (18). In addition, the 5' proximal end sequence does not code for a typical signal peptide sequence (see below).

The amino acid sequence predicted by nucleotide analysis is shown in Figures 1A and 1B. A protein data-base search revealed that the Der p I amino acid sequence showed homology with a group of cysteine proteases. Previous cDNA studies have shown that lysosomal cathepsins B, a mouse macrophage protease and a cysteine protease from an amoeba have transient pre- and proform intermediates (19-21), and inspection of the amino acid sequence at the 5' proximal end of the ggt11 p1 cDNA clone suggests that Der p I may be similar. First, the hydrophilicity plot (22) of the sequence preceding the mature protein sequence lacks the characteristic hydrophobic region of a signal peptide (23) and second, an Ala-X-Ala sequence, the most frequent sequence preceding the signal peptidase cleavage site (24,25), is present at positions -13, -14, -15 (Figures 1A and 1B). Therefore, it is proposed that cleavage between pro-Der p I sequence and the pre-Der p I sequence occurs between Ala (-13) and Phe (-12). Thus, pro-Der p I sequence begins at residues Phe (-12) and ends at residues Glu (-l). The amino acids residues numbered -13 to -23 would then correspond to a partial signal peptide sequence. The full length of the Der p I preproenzyme sequence has been

determined and is shown in Figures 21A and 21B. The negative sequence numbers refer to the pre- and preproenzyme forms of Der p I.

When the 857-bp cDNA insert was radiolabelled and hybridized against a Southern blot of EcoRI-digested genomic DNA from house dust mite, hybridization to bands of 1.5, 0.5, and 0.35 kb was observed (data not shown). As shown in the restriction enzyme map of the cDNA insert (Figure 2), there was no internal EcoRI site and the multiple hybridization bands observed suggest that Der p I is coded by a noncontiguous gene. The results also showed little evidence of gene duplication since hybridization was restricted to fragments with a total length of 2.4 kb.

The N-terminal can be compared with N-terminal of the equivalent protein from D.farinae (Der f I) (12). There is identity in 11/20 positions of the sequences available for comparison (Fig. 3).

To examine the protein produced by ggt11 p1(13T), phage was lysogenized in Y1089 (r-) and the bacteria grown in broth culture at 30°C. Phage was induced by temperature switch and isopropyl thiogalactopyranoside (IPTG) (6) and the bacteria were suspended in PBS to 1/20 of the culture volume, and sonicated for an antigen preparation. When examined by 7.5% SDS-PAGE electrophoresis it was found that ggt11 p1(13T) did not produce a Mr 116K ß-galactosidase band but instead produced a 140K band consistent with a fusion protein with the Der p I contributing a 24kDa moiety (6). Rabbit anti Der p I was shown to react with the lysate from ggt11 p1(13T) (Fig. 4).

## EXAMPLE 2

### Expression of Der p I cDNA products reactive with IgE from allergic serum.

The DNA insert from ggt11 p1(13T) which codes for Der p I was subcloned into the EcoRI site of the plasmid expression vector (pGEX)(26) where it could be expressed as a fusion with a glutathione transferase molecule. E. coli infected with this plasmid pGEX-p1(13T) or with the vector alone were grown to a log phase culture and harvested by centrifugation. The bacteria were suspended in PBS to 1/20 of their culture volume and lysed by freeze- thawing. The lysate was shown by sodium dodecyl- sulphate polyacrylamide electrophoresis to express a fusion protein in high concentration of the expected Mr 50,000. These lysates were then tested for their ability to react with IgE from allergic serum by radioimmune dot-blot conducted by the method described by Thomas and Rossi (27). The serum was taken from donors known to be mite-allergic or from non-allergic controls. Reactivity was developed by $^{125}$I-monoclonal anti-IgE and

autoradiography. Figure 5 shows the lysate from pGEX-p1(13T), but not the vector control reacted with IgE in allergic serum, but not non allergic serum.

## EXAMPLE 3

### Inhibition of IgE antibody responses to Der p I by treatment with the product from a cDNA clone coding for Der p I.

*E. coli* lysogenized by ggt11 p1(13T) were grown and induced by temperature switch to produce a recombinant fusion protein which was consistent with a 24 kD *Der p* I moiety and a 116 kD ß-galactosidase moiety (p1(13T) (28). This protein was mostly insoluble and could be isolated to about 90% purity, judged by sodium didodecyl polyacrylamide electrophoresis, by differential centrifugation. A similar protein was produced from another gt11 cDNA mite clone ggt pX (2c). To test for the ability of the recombinant protein to modify IgE antibody responses to *Der p* I, groups of 4-5 CBA mice were injected intraperitoneally with 2 mg of the p1(13T) or pX (2c) fusion proteins and after 2 days given a subcutaneous injection of 5mg of native *Der p* I (from mite culture medium) in aluminium hydroxide gel. The IgE antibody titres were measured by passive cutaneous anaphylaxis (PCA) after 3 and 6 weeks. The methods and background data for these responses have been described by Stewart and Holt (29). For a specificity control, groups of mice injected with p1(13T) or pX (2c) were also injected with 10mg of ovalbumin in alum. Responses were compared to mice without prior p1(13T) or pX (2c) treatment (Table 1). After 3 weeks mice either not given an injection of recombinant protein or injected with the control pX (2c) had detectable anti *Der p* I PCA titres (1/2 or greater). Only 1/5 of mice treated with recombinant p1(13T) had a detectable titre and this at 1/4 was lower than all of the titres of both control groups. Titres of all groups at 6 weeks were low or absent (not shown). The PCA response to ovalbumin was not significantly affected by treatment with recombinant proteins. These data show the potential of the recombinant proteins to specifically decrease IgE responses as required for a desensitizing agent.

TABLE 1 Inhibition of anti-Der p I IgE by preinjection with with recombinant Der p I.

| group | preinjection -2 days | immunizing injection (d0) (5mg/alum) | IgE (PCA) titres at d21 responders | titres |
|---|---|---|---|---|
| 1 | - | Der p I | 4/4 | 1/16-1/64 |
| 2 | pX(2C) | Der p I | 5/5 | 1/8-1/16 |
| 3 | p1(13T) | Der p I | 1/5* | 1/4* |
| 4 | - | ovalbumin | 4/4 | 1/64-1/256 |
| 5 | pX(2C) | ovalbumin | 5/5 | 1/32-1/128 |
| 6 | p1(13T) | ovalbumin | 5/5 | 1/64-1/256 |

Mice were given a preinjection on day -2 and then immunized with Der p I or ovalbumin on day 0. Serum antibody titres were measured on day 21 and 42 by PCA in rat skin. Significant anti-Der p I titres were not detected on day 42 (not shown). The PCA were measured to Der p I for groups 1-3 and ovalbumin for groups 4-6. The anti-Der p I titres were lower ($p<0.001$)* when pretreated with recombinant Der p I p1(13T).

*Mann Whitney analysis.

## EXAMPLE 4

Expression of Der p I antigenic determinants by fragments of the cDNA from ggt11 p1(13T)

The cDNA from ggt11 (13T) coding for Der p I was fragmented by sonication. The fragments (in varying size ranges) were isolated by electrophoresis. filled in by the Klenow reaction to create blunt ends. EcoRI linkers were attached and the fragment libraries cloned in ggt11. The methods used for the fragments cloning were the same as that used for cDNA cloning (6). Plaque immunoassay was used for screening with rabbit anti-Der p I. Three phage clones reacting with the antiserum were isolated and the oligonucleotide sequences of the cloned fragments obtained. Two of these were found to code for Der p I amino acids 17-55 (see Figures 1A and 1B for numbering) and one for amino acids 70-100. Such fragments will eventually be useful for both diagnostic reagents to

determine epitope reactivity and for therapy where molecules of limited allergenicity may increase safety of desensitisation.

## EXAMPLE 5

Cloning and expression of cDNA coding for the major mite allergen Der p II.

The Dermatophagoides pteronyssinus cDNA library in ggt11 previously described was screened by plaque radioimmune assay using nitrocellulose lifts (6). Instead of using specific antisera the sera used was from a person allergic to house dust mites. The serum (at 1/2 dilution) was absorbed with E. coli. To detect reactivity an $^{125}I$ labelled monoclonal anti-IgE was used (at 30ng/ml with $2x10^6$ cpm/ml (approx. 30% counting efficiency)). After 1 hour the filters were washed and autoradiography performed. Using this procedure 4 clones reacting with human IgE were isolated. It was found they were related by DNA hybridization and had an identical pattern of reactivity against a panel of allergic sera. Fig. 6 shows IgE reactivity in plaque radioimmunoassay against allergic serum (AM) (top row) or non allergic (WT). Here, clones 1, 3 and 8 react strongly, but only against allergic sera. The amp 1 segments (present in row 1) are a ggt11 vector control. The bottom row is an immunoassay with rabbit anti-Der p I, developed by $^{125}I$ staphylococcus protein A which shows no significant reactivity. The clones were tested against a panel of sera. Serum from five patients without allergy to mite did not react, but serum from 14/17 people with mite allergy showed reactivity. The DNA insert from the clone ggt11 pII(Cl) was subcloned into M13 mpl8 and M13 mp19 and sequenced by the chain termination method. The nucleotide sequence (Figures 7A and 7B) showed this allergen was Der p II by (a) the homology of the inferred amino acid sequence of residues 1-40 with that of the N-terminal amino acid of Der p II (30); and (b) the homology of this sequence with the equivalent Der f II allergen from Dermatophagoides farinae (30).

## EXAMPLE 6

Isolation and Characterization of cDNA Coding for Der f I

MATERIALS AND METHODS

Dermatophagoides farinae culture

Mites were purchased from Commonwealth Serum Laboratories, Parkville, Australia.

Construction of the D. farinae cDNA ggt11 library

Polyadenylated mRNA was isolated from live D. farinae mites and cDNA was synthesized by the RNase H method (Gubler, V. and B.J. Hoffman, Gene 25:263-269 (1983)) using a kit (Amersham International, Bucks.). After the addition of EcoRI linkers (New England Biolabs, Beverly, MA) the cDNA was ligated to alkaline phosphatase treated ggt11 arms (Promega, Madison, WI). The ligated DNA was packaged and plated in E. coli Y1090 (r-) to produce a library of $2x10^4$ recombinants.

Isolation of Der f I cDNA clones from the D. farinae cDNA ggt11 library

Screening of the library was performed by hybridization with two probes comprising the two Der p I cDNA BamHI fragments 1-348 and 349-857 generated by BamHI digestion of a derivative of the Der p I cDNA which has had two BamHI restriction sites inserted between amino acid residues -1 and 1 and between residues 116 and 117 by site-directed mutagenesis (Chua, K.Y. et al., J. Exp. Med. 167:175-182 (1988)). The probes were radiolabelled with $^{32}P$ by nick translation. Phage were plated at 20,000 pfu per 150mm petri dish and plaques were lifted onto nitrocellulose (Schleicher and Schull, Dassel, FRG), denatured and baked (Maniatis, T. et al., Molecular Cloning: Laboratory anual, Cold Spring Harbor Laboratory Press (1982)). Prehybridizations were performed for 2 hours at 42°C in 50% formamide/5 x SSCE/1 x Denhardt's/poly C (0.1mg/ml)/poly U(0.lmg/ml) with hybridization overnight at 42°C at $10^6$ cpm/ml. Post hybridization washes consisted of 15 min washes at room temperature with 2 x sodium chloride citrate (SSC)/0.1% sodium dodecylsulphate (SDS), 0.5 x SSC/0.1% SDS, 0.1 x SSC/0.1% SDS successively and a final wash at 50°C for 30 min in 0.1 x SSC/1% SDS.

Isolation of DNA from ggt11 f 1 cDNA clones

Phage DNA from ggt11 f 1 clones was prepared by a rapid isolation procedure. Clarified phage plate lysate (1 ml) was mixed with 270 of 25% wt/vol polyethylene glycol (PEG 6000) in 2.5M NaCl and incubated at room temperature for 15 min. The mixture was then spun for 5 min in a microfuge (Eppendorf, FRG), and the supernatant was removed. The pellet was dissolved in 100 mL of 10mM Tris/HC1 pH8.0 containing 1 mM EDTA and 100 mM NaCl (TE). This DNA preparation was extracted with phenol/TE, the phenol phase was washed with 100 mP TE, the pooled aqueous phases were then extracted another 2 times with phenol/TE, 2 times with Leder phenol (phenol/chloroform/isoamylalcohol; 25:24:1), once with chloroform and the DNA was precipitated by ethanol.

DNA sequencing

To obtain clones for DNA sequence analysis, the ggt11 fl phage DNA was digested with EcoRI restriction enzyme (Pharmacia, Uppsala, Sweden) and the DNA insert was ligated to EcoRI-digested M13-derived sequencing vectors mp18 and mp19 (Maniatis, T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1982)). Transformation was carried out using E. coli TG-1 and sequencing was performed by the dideoxynucleotide chain termination method (Sanger, F. et al., Proc. Natl. Acad. Sci. USA, 74:5463-5467 (1977)) using the Sequenase version 2.0 DNA sequencing kit (U.S.B., Cleveland, Ohio).

Polymerase chain reaction (PCR)

PCR was performed by the Taq DNA polymerase method (Saiki, R.K. et al., Science 239:487-491 (1988)) using the TaqPaq kit (Biotech International, Bentley, WA) and the conditions recommended by the supplier with 10ng of target DNA and 10pmol of ggt11 primers (New England BioLabs, Beverly, MA).

RESULTS

Isolation of Der f I cDNA clones

Two clones expressing the major mite allergen Der f I were isolated from the D. farinae cDNA ggt11 library by their ability to hybridize with both of the Der p I cDNA probes (nucleotides 1-348 and 349-857). This approach was adopted because amino acid sequencing had shown high homology (80%) between these two allergens (Thomas, W.R., et al., Advances in the Biosciences, 14:139-147 (1989)). Digestion of the ggt11 fl clone DNA with EcoRI restriction enzyme to release the cDNA insert produced three Der f I cDNA EcoRI fragments: one approximately 800 bases long and a doublet approximately 150 bases long. The Der f I cDNA insert was also amplified from the phage DNA by the polymerase chain reaction (PCR) resulting in a PCR product of approximately 1.1-kb. Each Der f I cDNA fragment was cloned separately into the M13-derived sequencing vectors mp18 and mp19 and sequenced.

DNA sequence analysis

The nucleotide sequence of Der f I cDNA was determined using the sequencing strategy shown in Figure 9. The complete sequence was shown to be 1084 bases long and included a 335-base long 5' proximal end sequence, a coding region for the entire native Der f I protein of 223 amino acids with a derived molecular weight of 25,191 and an 80-base long 3' noncoding region (Fig. 10).

The assignment of the threonine residue at position 1 as the $NH_2$-terminal amino acid of Der f I was based on data obtained by $NH_2$-terminal amino acid sequencing of the native protein and the predicted amino acid sequence of recombinant Der p I (Chua, K.Y. et al., J. Exp. Med., 167:175-182 (1988)). The predicted amino acid sequence of the Der f I cDNA in the $NH_2$-terminal region matched completely with that determined at the protein level (Figures 10A and 10B).

The complete mature protein coded by a single open reading frame terminating at the TGA stop codon at nucleotide position 42-44 is presumed to be the translation initiation site since the subsequent sequence codes for a typical signal peptide sequence.

Amino Acid Sequence Analysis

The amino acid sequence of Der f I predicted by nucleotide analysis is shown in Figures 10A and 10B. As shown in the composite alignment of the amino acid sequence of mature Der p I and Der f I (Figure 11), high homology was observed between the two proteins. Sequence homology analysis revealed that the Der f I protein showed 81% homology with the Der p I protein as predicted by previous conventional amino acid sequencing. In particular, the residues making up the active side of Der p I, based on those determined for papain, actinidin, cathepsin H, and cathepsin B, are also conserved in the Der f I protein. The residues are glutamine (residue 29), glycine, serine and cysteine (residues 33-35), histidine (residue 171) and asparagine, serine and tryptophan (residues 191-193) where the numbering refers to Der f I. The predicted mature Der f I amino acid sequence contains a potential N-glycosylation site (Asn-Thr-Ser) at position 53-55 which is also present as Asn-Gln-Ser at the equivalent position in Der p I.

Analysis of the predicted amino acid sequence of the entire Der f I cDNA insert has shown that, as for other cysteine proteases (Figures 12A and 12B), the Der f I protein has pre- and proform intermediates. As previously mentioned, the methionine residue at position -98 is presumed to be the initiation methionine. This assumption is based on the fact that firstly, the 5' proximal end sequence from residues -98 to -81 is composed predominantly of hydrophobic amino acid residues (72%), which is the characteristic feature of signal peptides (Von Heijne, G., EMBO J., 3:2315-2323 (1984)). Secondly, the lengths of the presumptive pre- (18 amino acid residues) and pro-peptides (80 residues) are similar to those for other cysteine proteases (Figures 12A and 12B). Most cysteine proteases examined have about 120 preproenzyme residues (of which an average of 19 residues form the signal peptide) with cathepsin B the smallest with 80 (Ishidoh, K. et al., FEBS Letters, 226:32-37 (1987)). Der f I falls within this range with a total of 98 preproenzyme residues.

By following the method for predicting signal-sequence cleavage sites outlined in Von Heijne, it is proposed that cleavage from the pre-Der f I sequence for proenzyme formation occurs at the signal peptidase cleavage site lying between Ala (-81) and Arg (-80) (Von Heijne, G., Eur. J. Biochem., 133:17-21 (1988) and J. Mol. Biol., 184:99-105 (1985)). Thus, the sequence from residues -98 to -81 codes for the leader peptide while the proenzyme moiety of Der f I begins at residue Arg (-80) and ends at residue Glu (-1).

## EXAMPLE 7

### Isolation and Characterization of cDNA Coding for Der f II

MATERIALS AND METHODS

Amino acid sequence analysis

Preparation of ggt11 D. farinae cDNA ligations

D. farinae was purchased from Commonwealth Serum Laboratories, Parkville, Australia, and used to prepare mRNA (polyadenylated RNA) as described (Stewart, G.A. and W.R. Thomas, Int. Arch. Allergy Appl Immunol., 83:384-389 (1987)). The mRNA was suspended at approximately 0.5mg/ml and 5mg used to prepare cDNA by the RNase H method (Gubler, U. and Hoffman, B.J., Gene, 25:263-269 (1983)) using a kit (Amersham International, Bucks). EcoRI linkers (Amersham, GGAATTCC) were attached according to the method described by Huynh et al., Constructing and screening cDNA libraries in gt10 and gt11, In: Glover, DNA Cloning vol. A practical approach pp. 47-78 IRL Press, Oxford (1985)). The DNA was then digested with EcoRI and recovered from an agarose gel purification by electrophoresis into a DEAE membrane (Schleicher and Schuell, Dassel, FRG, NA-45) according to protocol 6.24 of Sambrook et al., (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press (1989)), except 0.5M arginine base was used for elution. The cDNA was then ligated in ggtl0 and ggt11 at an arms to insert ratio of 2:1. Some was packaged for plaque libraries and an aliquot retained for isolating sequences by polymerase chain reaction as described below.

Isolation of Der f II cDNA by Polymerase Chain Reaction

To isolate Der f II cDNA, an oligonucleotide primer based on the N-terminal sequence of Der p II was made because their amino acid residues are identical in these regions (Heymann, P.W. et al., J. Allergy Clin. Immunol., 83:1055-1087 (1989)). The primer GGATCCGATCAACTCGATGC-3' was used. The first GGATCC encodes a BamH1 site and the following sequence GAT... encodes the first four residues of Der p II. For the other primer the ggt11 TTGACACCAGACCAACTGGTAATG-3' reverse primer flanking the EcoRI

cloning site was used (New England Biolabs, Beverly, MA). The Der p II primer was designed to have approximately 50-60% G-C and to end on the first or second, rather than the third, base of a codon (Gould, S.J. et al., Proc. Natl. Acad. Sci., 86:1934-1938 (1989); Summer, R. and D. Tautz, Nucleic Acid Res., 17:6749 (1989)).

The PCR reactions were carried out in a final reaction volume of 25 ml containing 67mM Tris-HCL (pH8.8 at 25°C), 16.6mM $(NH_4)_2SO_4$, 40mM dNTPs, 5mM 2-mercaptoethanol, 6mM EDTA, 0.2mg/ml gelatin, 2mM $MgCl_2$, 10pmoles of each primer and 2 units of Taq polymerase. Approximately 0.001mg of target DNA was added and the contents of the tube were mixed and overlayed with paraffin oil. The tubes were initially denatured at 95°C for 6 minutes, then annealed at 55°C for 1 minute and extended at 72°C for 2 minutes. Thereafter for 38 cycles, denaturing was carried out for 30 seconds and annealing and extension as before. In the final (40th) cycle, the extension reacton was increased to 10 minutes to ensure that all amplified products were full length. The annealing temperature was deliberately set slightly lower than the Tm of the oligonucleotide primers (determined by the formula Tm=69.3 + 0.41 (G+C%)-650/oligo length) to allow for mismatches in the N-terminal primer.

5ml of the reaction was then checked for amplified bands on a 1% agarose gel. The remainder of the reaction mixture was extracted with chloroform to remove all of the paraffin oil and ethanol precipitated prior to purification of the amplified product on a low melting point agarose gel (Bio-Rad, Richmond, CA).

Subcloning of PCR Product

The ends of the purified PCR product were filled in a reaction containing 10 mM Tris HC1, 10 mM $MgCl_2$, 50 mM NaCl, 0.025 mM dNTP and lml of Klenow enzyme in a final volume of 100ml. The reaction was carried out at 37°C for 15 minutes and heat inactivated at 70°C for 10 minutes. The mixture was Leder phenol extracted before ethanol precipitation. The resulting blunt ended DNA was ligated into M13mpl18 digested with Sma I in a reaction containing 0.5M ATP, 1 X ligase buffer and 1 unit of $T_4$ ligase at 15°C for 24 hrs and transformed into E. coli TG1 made competent by the $CaCl_2$ method. The transformed cells were plated out as a lawn on L + G plates and grown overnight at 37°C.

Preparation of Single-stranded DNA Template for Sequencing

Isolated white plaques were picked using an orange stick into 2.5 ml of an overnight culture of TG1 cells diluted 1 in 100 in 2 X TY broth, and grown at 37°C for 6 hours. The cultures were pelleted and the supernatant removed to a fresh tube. To a lml aliquot of this supernatant 270ml of 20% polyethylene glycol, 2.5M NaCl was added and the tube was vortexed before allowing it to stand at room temperature (RT) for 15 minutes. This was then spun down again and all traces of the supernatant were removed from the tube. The pellet was then resuspended in 100ml of 1 X TE buffer. At least 2 phenol:TE extractions were done, followed by 1 Leder phenol extraction and a $CHCP_3$ extraction. The DNA was precipitated in ethanol and resuspended in a final volume of 20ml of TE buffer.

DNA Analysis

DNA sequencing was performed with the dideoxynucleotide chain termination (Sanger, F. et al., Proc. Natl. Acad. Sci., 74:5463-5467 (1977)) using DNA produced from M13 derived vectors mp18 and mp19 in E. coli TG1 and T4 DNA polymerase (Sequenase version 2.0, USB Corp., Cleveland, Ohio; Restriction endonucleases were from Toyobo, (Osaka, Japan). All general procedures were by standard techniques (Sambrook, J. et al., A Laboratory Manual, 2d Ed. Cold Spring Harbor Laboratory Press (1989)). The sequence analysis was performed using the Mac Vector Software (IBI, New Haven, CT).

RESULTS

D. farinae cDNA ligated in ggt11 was used to amplify a sequence using an oligonucleotide primer with homology to nucleotides coding for the 4 N-terminal residues of Der p II and a reverse primer for the ggt11 sequence flanking the coding site. Two major bands of about 500 bp and 300 bp were obtained when the product was gel electrophoresed. These were ligated into M13 mp18 and a number of clones containing the 500 bp fragment were analyzed by DNA sequencing. Three clones produced sequence data from the N-terminal primer end and one from the other orientation. Where the sequence data from the two directions overlapped, a complete match was found. One of the clones read from the N-terminal primer, contained a one-base deletion which shifted the reading frame. It was deduced to be a copying error, as the translated sequence from the other two clones matched the protein sequence for the first 20 amino acid residues of the allergen.

The sequence of the clones showing consensus and producing a correct reading frame is shown in Figure 14, along with the inferred amino acid sequence. It coded for a 129 residue protein with no N-glycosylation site and a calculated molecular weight of 14,021 kD. No homology was found when compared to other proteins on the GenBank data base (61.0 release). It did, however, show 88% amino acid residue homology with Der p II shown in the alignment in Figures 16A, 16B, and 16C. Seven out of the 16 changes were conservative. The conserved residues also include all the cysteines present at positions 8, 21, 27, 73 and 119. There was also considerable nucleotide homology, although the restriction enzyme map generated from the sequence data for commonly used enzymes is different from Der p II (Figure 15). The hydrophobicity plots of the translated sequence of Der f II and Der p II shown in Figures 17A and 17B are almost identical.

## EXAMPLE 8

### Determination of Nucleotide Sequence Polymorphisms in the Der p I, Der p II and Der f II Allergens

It was expected that there were sequence polymorphisms in the nucleic acid sequence coding for Der p I, Der p II, Der f I and Der f II, due to natural allelic variation among individual mites. Several nucleotide and resulting amino acid sequence polymorphisms were discovered during the sequencing of different Der p I, Der p II and Der f II clones. The amino acid sequence polymorphisms are shown in Figures 18, 19 and 20.

The original Der p I ggt11 cDNA library was reprobed with cDNA obtained from the ggt11 pI(13T) clone to identify new clones. Similarly, the ggt11 cDNA library of Der p II was reprobed with cDNA obtained from the ggt11 pII(C1) clone to identify additional Der p II clones. These clones were isolated, sequenced and found to contain nucleotide and resulting amino acid sequence polymorphisms (see Fig. 18 and 19).

Four Der p I clones, (b), (c), (d) and (e) were sequenced, as shown in Fig. 18. Clone Der p I(d) was found to contain the following polymorphisms relative to the clone Der p I(a) sequence: (1) the codon for amino acid residue 136 was ACC rather than AGC, which results in a predicted amino acid substitution of Thr for Ser; (2) the codon for amino acid residue 149 had a silent mutation, GCT rather than GCA; and (3) the codon for amino acid residue 215 was CAA rather than GAA; which results in a predicted amino acid substitution of Gln for Glu.

The Der p II clones, Der p II(1) and Der p II(2) were sequenced as shown in Figure 19. Clone Der p II(2) was found to have the codon TCA, rather than ACA at amino acid residue 47, which results in a predicted amino acid substitution of Ser for Thr. This clone also was found to have the codon AAT at amino acid residue 113 rather than GAT, which results in a predicted amino acid substitution of Asn for Asp. The codon for amino acid 127 of this clone was found to be CTC rather than ATC. This change in codon 127 results in a predicted amino acid substitution of Leu for Ile.

Additional Der f II cDNA clones containing nucleic acid and resulting amino acid sequence polymorphisms were obtained from PCR reactions using cDNA prepared with RNA isolated from D. farinae mites (Commonwealth Serum Laboratories, Parksville, Australia). cDNA was prepared and ligated in ggt10 as previously described (Trudinger et al. (1991) Clin. Exp. Allergy 21:33-37). The clones described below were isolated following PCR of the ggt10 library using a 5' primer, which had the sequence 5'-GGATCCGATCAAGTCGATGT-3'. The nucleotides 5'-GGATCC-3' of the 5' primer correspond to a Bam HI endonuclease site added for cloning purposes. The remaining nucleotides of the 5' primer, 5'-GATCAAGTCGATGT-3' correspond to the first 4 amino acids of Der p II (Chua et al. (1990) Int. Arch. Allergy Clin. Immunol. 91:118-123) as described in Trudinger et al. ((1991) Clin. Exp. Allergy 21:33-37). The 3' primer, which has the sequence 5'-TTGACACCAGACCAACTGGTAATG-3', corresponds to a sequence of the ggt10 cloning vector (Trudinger et al. supra).

PCR was performed as described (Trudinger et al. supra) and four Der f II clones, MT3, MT5, MT16 and MT18, were sequenced, as shown in Figure 20. Three clones were sequenced that had potential polymorphisms relative to the published Der f II sequence (Trudinger et al. supra). The codon for amino acid 52 of clone MT18 was ATT rather than the published ACT (Trudinger et al. supra). This change in codon 52 of clone MT18 would result in a predicted amino acid change from Thr to Ile. Clone MT5 contained three changes from the published sequence (Trudinger et al. supra): (1) the codon for amino acid 11 was AGC rather than the published AAC (Trudinger et al. supra), which results in a predicted amino acid substitution of Ser for Asn; (2) the codon for amino acid 52 was ATT, rather than the published ACT (Trudinger et al. supra), which results in a predicted amino acid substitution of Ile for Thr; and (3) the codon for amino acid 88 was ATC rather than the published GCC (Trudinger et al. supra), which results in a predicted amino acid substitution of Ile for Ala. Clone MT16 had a

silent mutation in the codon for amino acid 68 (ATC versus the published ATT (Trudinger et al. supra) that did not change the predicted amino acid at this residue. The following substitutes were also observed by Yuuki et al. (Jpn.J.Allergol. 6:557-561, 1990); Ile at residue 52, Ile at residue 54 and Ile at residue 88.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: ASCII TEXT

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/945,288

(B) FILING DATE: 10 SEPTEMBER 1992

(ix) TELECOMMUNICATION INFORMATION:

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(B) TELEFAX: (617) 227-5941

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 834 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..738

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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-23         -20                 -15                 -10

CAA TTC GAT TTG AAT GCT GAA ACT AAC GCC TGC AGT ATC AAT GGA AAT        96
Gln Phe Asp Leu Asn Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn
        -5                  -1   1               5
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GCT CCA GCT GAA ATC GAT TTG CGA CAA ATG CGA ACT GTC ACT CCC ATT 144
Ala Pro Ala Glu Ile Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile
10 15 20 25

CGT ATG CAA GGA GGC TGT GGT TCA TGT TGG GCT TTC TCT GGT GTT GCC 192
Arg Met Gln Gly Gly Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala
30 35 40

GCA ACT GAA TCA GCT TAT TTG GCT CAC CGT AAT CAA TCA TTG GAT CTT 240
Ala Thr Glu Ser Ala Tyr Leu Ala His Arg Asn Gln Ser Leu Asp Leu
45 50 55

GCT GAA CAA GAA TTA GTC GAT TGT GCT TCC CAA CAC GGT TGT CAT GGT 288
Ala Glu Gln Glu Leu Val Asp Cys Ala Ser Gln His Gly Cys His Gly
60 65 70

GAT ACC ATT CCA CGT GGT ATT GAA TAC ATC CAA CAT AAT GGT GTC GTC 336
Asp Thr Ile Pro Arg Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val
75 80 85

CAA GAA AGC TAC TAT CGA TAC GTT GCA CGA GAA CAA TCA TGC CGA CGA 384
Gln Glu Ser Tyr Tyr Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg
90 95 100 105

CCA AAT GCA CAA CGT TTC GGT ATC TCA AAC TAT TGC CAA ATT TAC CCA 432
Pro Asn Ala Gln Arg Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro
110 115 120

CCA AAT GCA AAC AAA ATT CGT GAA GCT TTG GCT CAA ACC CAC AGC GCT 480
Pro Asn Ala Asn Lys Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala
125 130 135

ATT GCC GTC ATT ATT GGC ATC AAA GAT TTA GAC GCA TTC CGT CAT TAT 528
Ile Ala Val Ile Ile Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr
140 145 150

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GAT GGC CGA ACA ATC ATT CAA CGC GAT AAT GGT TAC CAA CCA AAC TAT     576
Asp Gly Arg Thr Ile Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr
    155                 160                 165

CAC GCT GTC AAC ATT GTT GGT TAC AGT AAC GCA CAA GGT GTC GAT TAT     624
His Ala Val Asn Ile Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr
170                 175                 180                 185

TGG ATC GTA CGA AAC AGT TGG GAT ACC AAT TGG GGT GAT AAT GGT TAC     672
Trp Ile Val Arg Asn Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr
                190                 195                 200

GGT TAT TTT GCT GCC AAC ATC GAT TTG ATG ATG ATT GAA GAA TAT CCA     720
Gly Tyr Phe Ala Ala Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro
            205                 210                 215

TAT GTT GTC ATT CTC TAAACAAAAA GACAATTTCT TATATGATTG TCACTAATTT     775
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        220

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 245 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Gln Phe Asp Leu Asn Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn
-5 -1 1 5

Ala Pro Ala Glu Ile Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile
10 15 20 25

Arg Met Gln Gly Gly Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala
30 35 40

Ala Thr Glu Ser Ala Tyr Leu Ala His Arg Asn Gln Ser Leu Asp Leu
45 50 55

Ala Glu Gln Glu Leu Val Asp Cys Ala Ser Gln His Gly Cys His Gly
60 65 70

Asp Thr Ile Pro Arg Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val
75 80 85

Gln Glu Ser Tyr Tyr Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg
90 95 100 105

Pro Asn Ala Gln Arg Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro
110 115 120

Pro Asn Ala Asn Lys Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala
125 130 135

Ile Ala Val Ile Ile Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr
140 145 150

Asp Gly Arg Thr Ile Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr
155 160 165

His Ala Val Asn Ile Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr
170 175 180 185

Trp Ile Val Arg Asn Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr
190 195 200

Gly Tyr Phe Ala Ala Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro
205 210 215

Tyr Val Val Ile Leu
220

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 588 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 69..509

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CACAAATTCT TCTTTCTTCC TTACTACTGA TCATTAATCT GAAAACAAAA CCAAACAAAC      60

CATTCAAA ATG ATG TAC AAA ATT TTG TGT CTT TCA TTG TTG GTC GCA GCC      110
             Met Tyr Lys Ile Leu Cys Leu Ser Leu Leu Val Ala Ala
             -16 -15                 -10                     -5

GTT GCT CGT GAT CAA GTC GAT GTC AAA GAT TGT GCC AAT CAT GAA ATC      158
Val Ala Arg Asp Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile
         -1   1               5                  10

AAA AAA GTT TTG GTA CCA GGA TGC CAT GGT TCA GAA CCA TGT ATC ATT      206
Lys Lys Val Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile
     15                  20                  25

CAT CGT GGT AAA CCA TTC CAA TTG GAA GCC GTT TTC GAA GCC AAC CAA      254
His Arg Gly Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln
 30                  35                  40                  45

AAC ACA AAA ACG GCT AAA ATT GAA ATC AAA GCC TCA ATC GAT GGT TTA      302
Asn Thr Lys Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu
                 50                  55                  60

GAA GTT GAT GTT CCC GGT ATC GAT CCA AAT GCA TGC CAT TAC ATG AAA      350
Glu Val Asp Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys
             65                  70                  75

TGC CCA TTG GTT AAA GGA CAA CAA TAT GAT ATT AAA TAT ACA TGG AAT      398
Cys Pro Leu Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn
         80                  85                  90
```

GTT CCG AAA ATT GCA CCA AAA TCT GAA AAT GTT GTC GTC ACT GTT AAA 446
Val Pro Lys Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys
95 100 105

GTT ATG GGT GAT GAT GGT GTT TTG GCC TGT GCT ATT GCT ACT CAT GCT 494
Val Met Gly Asp Asp Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala
110 115 120 125

AAA ATC CGC GAT TAAATAAACA AAATTTATTG ATTTTGTAAT CACAAATGAT 546
Lys Ile Arg Asp

TGATTTTCTT TCCAAAAAAA AAATAAATAA AATTTTGGGA AT 588

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Met Tyr Lys Ile Leu Cys Leu Ser Leu Leu Val Ala Ala Val Ala
-16 -15 -10 -5

Arg Asp Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile Lys Lys
-1 1 5 10 15

Val Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile His Arg
20 25 30

Gly Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln Asn Thr
35 40 45

Lys Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu Glu Val
50 55 60

Asp Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys Pro
65 70 75

Leu Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val Pro
80 85 90 95

Lys Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Val Met
100 105 110

Gly Asp Asp Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile
115 120 125

Arg Asp

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1072 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 36..1001

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CGTTTTCTTC CATCAAAATT AAAAATTCAT CAAAA ATG AAA TTC GTT TTG GCC       53
                                       Met Lys Phe Val Leu Ala
                                       -98         -95

ATT GCC TCT TTG TTG GTA TTG AGC ACT GTT TAT GCT CGT CCA GCT TCA     101
Ile Ala Ser Leu Leu Val Leu Ser Thr Val Tyr Ala Arg Pro Ala Ser
        -90                 -85                 -80

ATC AAA ACT TTT GAA GAA TTC AAA AAA GCC TTC AAC AAA AAC TAT GCC     149
Ile Lys Thr Phe Glu Glu Phe Lys Lys Ala Phe Asn Lys Asn Tyr Ala
    -75                 -70                 -65

ACC GTT GAA GAG GAA GAA GTT GCC CGT AAA AAC TTT TTG GAA TCA TTG     197
Thr Val Glu Glu Glu Glu Val Ala Arg Lys Asn Phe Leu Glu Ser Leu
-60                 -55                 -50                 -45

AAA TAT GTT GAA GCT AAC AAA GGT GCC ATC AAC CAT TTG TCC GAT TTG     245
Lys Tyr Val Glu Ala Asn Lys Gly Ala Ile Asn His Leu Ser Asp Leu
                -40                 -35                 -30

TCA TTG GAT GAA TTC AAA AAC CGT TAT TTG ATG AGT GCT GAA GCT TTT     293
Ser Leu Asp Glu Phe Lys Asn Arg Tyr Leu Met Ser Ala Glu Ala Phe
            -25                 -20                 -15

GAA CAA CTC AAA ACT CAA TTC GAT TTG AAT GCC GAA ACA AGC GCT TGC     341
Glu Gln Leu Lys Thr Gln Phe Asp Leu Asn Ala Glu Thr Ser Ala Cys
        -10                 -5                  -1   1
```

```
CGT ATC AAT TCG GTT AAC GTT CCA TCG GAA TTG GAT TTA CGA TCA CTG   389
Arg Ile Asn Ser Val Asn Val Pro Ser Glu Leu Asp Leu Arg Ser Leu
  5                  10                  15                  20

CGA ACT GTC ACT CCA ATC CGT ATG CAA GGA GGC TGT GGT TCA TGT TGG   437
Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly Cys Gly Ser Cys Trp
                 25                  30                  35

GCT TTC TCT GGT GTT GCC GCA ACT GAA TCA GCT TAT TTG GCC TAC CGT   485
Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala Tyr Leu Ala Tyr Arg
             40                  45                  50

AAC ACG TCT TTG GAT CTT TCT GAA CAG GAA CTC GTC GAT TGC GCA TCT   533
Asn Thr Ser Leu Asp Leu Ser Glu Gln Glu Leu Val Asp Cys Ala Ser
         55                  60                  65

CAA CAC GGA TGT CAC GGC GAT ACA ATA CCA AGA GGC ATC GAA TAC ATC   581
Gln His Gly Cys His Gly Asp Thr Ile Pro Arg Gly Ile Glu Tyr Ile
     70                  75                  80

CAA CAA AAT GGT GTC GTT GAA GAA AGA AGC TAT CCA TAC GTT GCA CGA   629
Gln Gln Asn Gly Val Val Glu Glu Arg Ser Tyr Pro Tyr Val Ala Arg
 85                  90                  95                 100

GAA CAA CGA TGC CGA CGA CCA AAT TCG CAA CAT TAC GGT ATC TCA AAC   677
Glu Gln Arg Cys Arg Arg Pro Asn Ser Gln His Tyr Gly Ile Ser Asn
                105                 110                 115

TAC TGC CAA ATT TAT CCA CCA GAT GTG AAA CAA ATC CGT GAA GCT TTG   725
Tyr Cys Gln Ile Tyr Pro Pro Asp Val Lys Gln Ile Arg Glu Ala Leu
            120                 125                 130
```

```
ACT CAA ACA CAC ACA GCT ATT GCC GTC ATT ATT GGC ATC AAA GAT TTG        773
Thr Gln Thr His Thr Ala Ile Ala Val Ile Ile Gly Ile Lys Asp Leu
        135                 140                 145

AGA GCT TTC CAA CAT TAT GAT GGA CGA ACA ATC ATT CAA CAT GAC AAT        821
Arg Ala Phe Gln His Tyr Asp Gly Arg Thr Ile Ile Gln His Asp Asn
    150                 155                 160

GGT TAT CAA CCA AAC TAT CAT GCC GTC AAC ATT GTC GGT TAC GGA AGT        869
Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile Val Gly Tyr Gly Ser
165                 170                 175                 180

ACA CAA GGC GAC GAT TAT TGG ATC GTA CGA AAC AGT TGG GAT ACT ACC        917
Thr Gln Gly Asp Asp Tyr Trp Ile Val Arg Asn Ser Trp Asp Thr Thr
                185                 190                 195

TGG GGA GAT AGC GGA TAC GGA TAT TTC CAA GCC GGA AAC AAC CTC ATG        965
Trp Gly Asp Ser Gly Tyr Gly Tyr Phe Gln Ala Gly Asn Asn Leu Met
            200                 205                 210

ATG ATC GAA CAA TAT CCA TAT GTT GTA ATC ATG TGAACATTTG AAATTGAATA     1018
Met Ile Glu Gln Tyr Pro Tyr Val Val Ile Met
        215                 220

TATTTATTTG TTTTCAAAAT AAAAACAACT ACTCTTGCGA GTATTTTTTA CTCG           1072
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Lys Phe Val Leu Ala Ile Ala Ser Leu Leu Val Leu Ser Thr Val
-98         -95                     -90                     -85

Tyr Ala Arg Pro Ala Ser Ile Lys Thr Phe Glu Glu Phe Lys Lys Ala
        -80                 -75                 -70

Phe Asn Lys Asn Tyr Ala Thr Val Glu Glu Glu Glu Val Ala Arg Lys
    -65                 -60                 -55

Asn Phe Leu Glu Ser Leu Lys Tyr Val Glu Ala Asn Lys Gly Ala Ile
-50                 -45                 -40                 -35

Asn His Leu Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Tyr Leu
                -30                 -25                 -20

Met Ser Ala Glu Ala Phe Glu Gln Leu Lys Thr Gln Phe Asp Leu Asn
            -15                 -10                 -5

Ala Glu Thr Ser Ala Cys Arg Ile Asn Ser Val Asn Val Pro Ser Glu
    -1   1              5                   10

Leu Asp Leu Arg Ser Leu Arg Thr Val Thr Pro Ile Arg Met Gln Gly
 15                 20                  25                  30

Gly Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser
                35                  40                  45

Ala Tyr Leu Ala Tyr Arg Asn Thr Ser Leu Asp Leu Ser Glu Gln Glu
            50                  55                  60
```

Leu Val Asp Cys Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro
65 70 75

Arg Gly Ile Glu Tyr Ile Gln Gln Asn Gly Val Val Glu Glu Arg Ser
80 85 90

Tyr Pro Tyr Val Ala Arg Glu Gln Arg Cys Arg Arg Pro Asn Ser Gln
95 100 105 110

His Tyr Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asp Val Lys
115 120 125

Gln Ile Arg Glu Ala Leu Thr Gln Thr His Thr Ala Ile Ala Val Ile
130 135 140

Ile Gly Ile Lys Asp Leu Arg Ala Phe Gln His Tyr Asp Gly Arg Thr
145 150 155

Ile Ile Gln His Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn
160 165 170

Ile Val Gly Tyr Gly Ser Thr Gln Gly Asp Asp Tyr Trp Ile Val Arg
175 180 185 190

Asn Ser Trp Asp Thr Thr Trp Gly Asp Ser Gly Tyr Gly Tyr Phe Gln
195 200 205

Ala Gly Asn Asn Leu Met Met Ile Glu Gln Tyr Pro Tyr Val Val Ile
210 215 220

Met

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..390

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GAT CAA GTC GAT GTT AAA GAT TGT GCC AAC AAT GAA ATC AAA AAA GTA       48
Asp Gln Val Asp Val Lys Asp Cys Ala Asn Asn Glu Ile Lys Lys Val
  1               5                  10                  15

ATG GTC GAT GGT TGC CAT GGT TCT GAT CCA TGC ATA ATC CAT CGT GGT       96
Met Val Asp Gly Cys His Gly Ser Asp Pro Cys Ile Ile His Arg Gly
                 20                  25                  30

AAA CCA TTC ACT TTG GAA GCC TTA TTC GAT GCC AAC CAA AAC ACT AAA      144
Lys Pro Phe Thr Leu Glu Ala Leu Phe Asp Ala Asn Gln Asn Thr Lys
             35                  40                  45

ACC GCT AAA ACT GAA ATC AAA GCC AGC CTC GAT GGT CTT GAA ATT GAT      192
Thr Ala Lys Thr Glu Ile Lys Ala Ser Leu Asp Gly Leu Glu Ile Asp
         50                  55                  60
```

```
GTT CCC GGT ATT GAT ACC AAT GCT TGC CAT TTT ATG AAA TGT CCA TTG      240
Val Pro Gly Ile Asp Thr Asn Ala Cys His Phe Met Lys Cys Pro Leu
 65                 70                  75                  80

GTT AAA GGT CAA CAA TAT GAT GCC AAA TAT ACA TGG AAT GTG CCC AAA      288
Val Lys Gly Gln Gln Tyr Asp Ala Lys Tyr Thr Trp Asn Val Pro Lys
                85                  90                  95

ATT GCA CCA AAA TCT GAA AAC GTT GTC GTT ACA GTC AAA CTT GTT GGT      336
Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Leu Val Gly
            100                 105                 110

GAT AAT GGT GTT TTG GCT TGC GCT ATT GCT ACC CAC GCT AAA ATC CGT      384
Asp Asn Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile Arg
        115                 120                 125

GAT TAAAAAAAAA AAATAAATAT GAAAATTTTC ACCAACATCG AACAAAATTC           437
Asp
    130

AATAACCAAA ATTTGAATCA AAAACGGAAT TCCAAGCTGA GCGCCGGTCG CTAC          491
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 129 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Asp Gln Val Asp Val Lys Asp Cys Ala Asn Asn Glu Ile Lys Lys Val
1               5                   10                  15

Met Val Asp Gly Cys His Gly Ser Asp Pro Cys Ile Ile His Arg Gly
            20                  25                  30

Lys Pro Phe Thr Leu Glu Ala Leu Phe Asp Ala Asn Gln Asn Thr Lys
        35                  40                  45

Thr Ala Lys Thr Glu Ile Lys Ala Ser Leu Asp Gly Leu Glu Ile Asp
    50                  55                  60

Val Pro Gly Ile Asp Thr Asn Ala Cys His Phe Met Lys Cys Pro Leu
65                  70                  75                  80

Val Lys Gly Gln Gln Tyr Asp Ala Lys Tyr Thr Trp Asn Val Pro Lys
                85                  90                  95

Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Leu Val Gly
            100                 105                 110

Asp Asn Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile Arg
        115                 120                 125

Asp
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1172 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..738

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GAATTCCTTT TTTTTTCTTT CTCTCTCTAA AATCTAAAAT CCATCCAAC ATG AAA ATT      58
                                                       Met Lys Ile
                                                       -98

GTT TTG GCC ATC GCC TCA TTG TTG GCA TTG AGC GCT GTT TAT GCT CGT      106
Thr Leu Ala Ile Ala Ser Leu Leu Ala Leu Ser Ala Val Tyr Ala Arg
-95                 -90                 -85                 -80

CCA TCA TCG ATC AAA ACT TTT GAA GAA TAC AAA AAA GCC TTC AAC AAA      154
Pro Ser Ser Ile Lys Thr Phe Glu Glu Tyr Lys Lys Ala Phe Asn Lys
                -75                 -70                 -65

AGT TAT GCT ACC TTC GAA GAT CAA GAA GCT GCC CGT AAA AAC TTT TTG      202
Ser Tyr Ala Thr Phe Glu Asp Glu Glu Ala Ala Arg Lys Asn Phe Leu
            -60                 -55                 -50
```

```
GAA TCA GTA AAA TAT GTT CAA TCA AAT GGA GGT GCC ATC AAC CAT TTG    250
Glu Ser Val Lys Tyr Val Gln Ser Asn Gly Gly Ala Ile Asn His Leu
        -45                 -40                 -35

TCC GAT TTG TCG TTG GAT GAA TTC AAA AAC CGA TTT TTG ATG AGT GCA    298
Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Phe Leu Met Ser Ala
    -30                 -25                 -20

GAA GCT TTT GAA CAC CTC AAA ACT CAA TTC GAT TTG AAT GCT GAA ACT    346
Glu Ala Phe Glu His Leu Lys Thr Gln Phe Asp Leu Asn Ala Glu Thr
-15                 -10                 -5                  -1   1

AAC GCC TGC AGT ATC AAT GGA AAT GCT CCA GCT GAA ATC GAT TTG CGA    394
Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile Asp Leu Arg
                5                   10                  15

CAA ATG CGA ACT GTC ACT CCC ATT CGT ATG CAA GGA GGC TGT GGT TCA    442
Gln Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly Cys Gly Ser
        20                  25                  30

TGT TGG GCT TTC TCT GGT GTT GCC GCA ACT GAA TCA GCT TAT TTG GCT    490
Cys Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala Tyr Leu Ala
    35                  40                  45

CAC CGT AAT CAA TCA TTG GAT CTT GCT GAA CAA GAA TTA GTC GAT TGT    538
His Arg Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu Val Asp Cys
50                  55                  60                  65

GCT TCC CAA CAC GGT TGT CAT GGT GAT ACC ATT CCA CGT GGT ATT GAA    586
Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg Gly Ile Glu
                70                  75                  80

TAC ATC CAA CAT AAT GGT GTC GTC CAA GAA AGC TAC TAT CGA TAC GTT    634
Tyr Ile Gln His Asn Gly Val Val Gln Glu Ser Tyr Tyr Arg Tyr Val
            85                  90                  95
```

GCA CGA GAA CAA TCA TGC CGA CGA CCA AAT GCA CAA CGT TTC GGT ATC 682
Ala Arg Glu Gln Ser Cys Arg Arg Pro Asn Ala Gln Arg Phe Gly Ile
100 105 110

TCA AAC TAT TGC CAA ATT TAC CCA CCA AAT GCA AAC AAA ATT CGT GAA 730
Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn Ala Asn Lys Ile Arg Glu
115 120 125

GCT TTG GCT CAA ACC CAC AGC GCT ATT GCC GTC ATT ATT GGC ATC AAA 778
Ala Leu Ala Gln Thr His Ser Ala Ile Ala Val Ile Ile Gly Ile Lys
130 135 140 145

GAT TTA GAC GCA TTC CGT CAT TAT GAT GGC CGA ACA ATC ATT CAA CGC 826
Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile Ile Gln Arg
150 155 160

GAT AAT GGT TAC CAA CCA AAC TAT CAC GCT GTC AAC ATT GTT GGT TAC 874
Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile Val Gly Tyr
165 170 175

AGT AAC GCA CAA GGT GTC GAT TAT TGG ATC GTA CGA AAC AGT TGG GAT 922
Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn Ser Trp Asp
180 185 190

ACC AAT TGG GGT GAT AAT GGT TAC GGT TAT TTT GCT GCC AAC ATC GAT 970
Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr Phe Ala Ala Asn Ile Asp
195 200 205

TTG ATG ATG ATT GAA GAA TAT CCA TAT GTT GTC ATT CTC TAAACAAAAA 1019
Leu Met Met Ile Glu Glu Tyr Pro Tyr Val Val Ile Leu
210 215 220

GACAATTTCT TATATGATTG TCACTAATTT ATTTAAAATC AAAATTTTTA GAAAATGAAT 1079

AAATTCATTC ACAAAAATTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1139

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA 1172

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 320 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Lys Ile Thr Leu Ala Ile Ala Ser Leu Leu Ala Leu Ser Ala Val
-98         -95                     -90                 -85

Tyr Ala Arg Pro Ser Ser Ile Lys Thr Phe Glu Glu Tyr Lys Lys Ala
        -80                 -75                 -70

Phe Asn Lys Ser Tyr Ala Thr Phe Glu Asp Glu Glu Ala Ala Arg Lys
    -65                 -60                 -55

Asn Phe Leu Glu Ser Val Lys Tyr Val Gln Ser Asn Gly Gly Ala Ile
-50                 -45                 -40                 -35

Asn His Leu Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Phe Leu
                -30                 -25                 -20

Met Ser Ala Glu Ala Phe Glu His Leu Lys Thr Gln Phe Asp Leu Asn
            -15                 -10                 -5
```

Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile
-1 1 5 10

Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly
15 20 25 30

Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala
35 40 45

Tyr Leu Ala His Arg Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu
50 55 60

Val Asp Cys Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg
65 70 75

Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val Gln Glu Ser Tyr Tyr
80 85 90

Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg Pro Asn Ala Gln Arg
95 100 105 110

Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn Ala Asn Lys
115 120 125

Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala Ile Ala Val Ile Ile
130 135 140

Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile
145 150 155

Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile
160 165 170

Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn
175 180 185 190

Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr Phe Ala Ala
195 200 205

Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro Tyr Val Val Ile Leu
210 215 220

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 222 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 50

(D) OTHER INFORMATION: /label=Xaa is His or Tyr

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 81

(D) OTHER INFORMATION: /label=Xaa is Glu or Lys

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 124

(D) OTHER INFORMATION: /label=Xaa is Ala or Val

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 136

(D) OTHER INFORMATION: /label=Xaa is Ser or Thr

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 215

(D) OTHER INFORMATION: /label=Xaa is Glu or Gln

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Thr Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile Asp Leu
 1               5                  10                  15

Arg Gln Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly Cys Gly
            20                  25                  30

Ser Cys Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala Tyr Leu
        35                  40                  45

Ala Xaa Arg Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu Val Asp
    50                  55                  60

Cys Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg Gly Ile
65                  70                  75                  80

Xaa Tyr Ile Gln His Asn Gly Val Val Gln Glu Ser Tyr Tyr Arg Tyr
                85                  90                  95

Val Ala Arg Glu Gln Ser Cys Arg Arg Pro Asn Ala Gln Arg Phe Gly
            100                 105                 110
```

```
Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn Xaa Asn Lys Ile Arg
        115                 120                 125

Glu Ala Leu Ala Gln Thr His Xaa Ala Ile Ala Val Ile Ile Gly Ile
    130                 135                 140

Lys Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile Ile Gln
145                 150                 155                 160

Arg Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile Val Gly
                165                 170                 175

Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn Ser Trp
            180                 185                 190

Asp Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr Phe Ala Ala Asn Ile
        195                 200                 205

Asp Leu Met Met Ile Glu Xaa Tyr Pro Tyr Val Val Ile Leu
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 47

(D) OTHER INFORMATION: /label=Xaa is Thr or Ser

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 114

(D) OTHER INFORMATION: /label=Xaa is Asp or Asn

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 127

(D) OTHER INFORMATION: /label=Xaa is Ile or Leu

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile Lys Lys Val
1 5 10 15

Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile His Arg Gly
20 25 30

Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln Asn Xaa Lys
35 40 45

Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu Glu Val Asp
50 55 60

Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys Pro Leu
65 70 75 80

Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val Pro Lys
85 90 95

Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Val Met Gly
100 105 110

Asp Xaa Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Xaa Arg
115 120 125

Asp

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 11
(D) OTHER INFORMATION: /label=Xaa is Asn or Ser

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 52
(D) OTHER INFORMATION: /label=Xaa is Thr or Ile

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 54
(D) OTHER INFORMATION: /label=Xaa is Ile or Thr

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 76
(D) OTHER INFORMATION: /label=Xaa is Met or Val

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 88
(D) OTHER INFORMATION: /label=Xaa is Ala or Ile

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 111
(D) OTHER INFORMATION: /label=Xaa is Val or Ile

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Asp Gln Val Asp Val Lys Asp Cys Ala Asn Xaa Glu Ile Lys Lys Val
  1               5                  10                  15

Met Val Asp Gly Cys His Gly Ser Asp Pro Cys Ile Ile His Arg Gly
             20                  25                  30

Lys Pro Phe Thr Leu Glu Ala Leu Phe Asp Ala Asn Gln Asn Thr Lys
         35                  40                  45

Thr Ala Lys Xaa Glu Xaa Lys Ala Ser Leu Asp Gly Leu Glu Ile Asp
     50                  55                  60

Val Pro Gly Ile Asp Thr Asn Ala Cys His Phe Xaa Lys Cys Pro Leu
 65                  70                  75                  80

Val Lys Gly Gln Gln Tyr Asp Xaa Lys Tyr Thr Trp Asn Val Pro Lys
                 85                  90                  95
```

```
Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Leu Xaa Gly
            100                 105                 110

Asp Asn Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile Arg
        115                 120                 125

Asp
```

CLAIMS

1. A protein allergen of Der p II comprising the amino acid sequence:

Asp Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile Lys Lys Val Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile His Arg Gly Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln Asn $Xaa_1$ Lys Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu Glu Val Asp Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys Pro Leu Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val Pro Lys Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Val Met Gly $Xaa_2$ Asp Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys $Xaa_3$ Arg Asp

where $Xaa_1$ is selected from the group consisting of Thr and Ser;
where $Xaa_2$ is selected from the group consisting of Asp and Asn; and
where $Xaa_3$ is selected from the group consisting of Ile and Leu, except for the amino acid sequence where $Xaa_1$ is Thr, $Xaa_2$ is Asp and $Xaa_3$ is Ile.

2. A protein allergen of Der f II comprising the amino acid sequence:

Asp Gln Val Asp Val Lys Asp Cys Ala Asn $Xaa_1$ Glu Ile Lys Lys Val Met Val Asp Gly Cys His Gly Ser Asp Pro Cys Ile Ile His Arg Gly Lys Pro Phe Thr Leu Glu Ala Leu Phe Asp Ala Asn Gln Asn Thr Lys Thr Ala Lys $Xaa_2$ Glu $Xaa_3$ Lys Ala Ser Leu Asp Gly Leu Glu Ile Asp Val Pro Gly Ile Asp Thr Asn Ala Cys His Phe $Xaa_4$ Lys Cys Pro Leu Val Lys Gly Gln Gln Tyr Asp $Xaa_5$ Lys Tyr Thr Trp Asn Val Pro Lys Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Leu $Xaa_6$ Gly Asp Asn Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile Arg Asp

where $Xaa_1$ is selected from the group consisting of Asn and Ser;
where $Xaa_2$ is selected from the group consisting of Thr and Ile;
where $Xaa_3$ is selected from the group consisting of Ile and Thr;
where $Xaa_4$ is selected from the group consisting of Met and Val;
where $Xaa_5$ is selected from the group consisting of Ala and Ile; and
where $Xaa_6$ is selected from the group consisting of Val and Ile, with the proviso that,

when $Xaa_1$ is Asn, then $Xaa_3$ is Thr; and
when $Xaa_3$ is Ile, then $Xaa_1$ is Ser.

3. A therapeutic composition comprising a protein allergen of claim 1 and a pharmaceutically acceptable carrier or diluent.

4. A method of treatment for sensitivity in an individual to house dust mites, comprising administering to the individual an effective therapeutic amount of a composition of claim 3.

5. A therapeutic composition comprising a protein allergen of claim 2 and a pharmaceutically acceptable carrier or diluent.

6. A method of treatment for sensitivity in an individual to house dust mites, comprising administering to the individual an effective therapeutic amount of a composition of claim 5.

```
-23                                                          -10
AAA AAC CGA TTT TTG ATG AGT GCA GAA GCT TTT GAA CAC CTC AAA ACT  48
Lys Asn Arg Phe Leu Met Ser Ala Glu Ala Phe Glu His Leu Lys Thr
                         -1   1
CAA TTC GAT TTG AAT GCT GAA ACT AAC GCC TGC AGT ATC AAT GGA AAT  96
Gln Phe Asp Leu Asn Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn
 10                                     20
GCT CCA GCT GAA ATC GAT TTG CGA CAA ATG CGA ACT GTC ACT CCC ATT 144
Ala Pro Ala Glu Ile Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile
                 30                                      40
CGT ATG CAA GGA GGC TGT GGT TCA TGT TGG GCT TTC TCT GGT GTT GCC 192
Arg Met Gln Gly Gly Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala
                                 50
GCA ACT GAA TCA GCT TAT TTG GCT CAC CGT AAT CAA TCA TTG GAT CTT 240
Ala Thr Glu Ser Ala Tyr Leu Ala His Arg Asn Gln Ser Leu Asp Leu
         60                                      70
GCT GAA CAA GAA TTA GTC GAT TGT GCT TCC CAA CAC GGT TGT CAT GGT 288
Ala Glu Gln Glu Leu Val Asp Cys Ala Ser Gln His Gly Cys His Gly
                         80
GAT ACC ATT CCA CGT GGT ATT GAA TAC ATC CAA CAT AAT GGT GTC GTC 336
Asp Thr Ile Pro Arg Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val
 90                                     100
CAA GAA AGC TAC TAT CGA TAC GTT GCA CGA GAA CAA TCA TGC CGA CGA 384
Gln Glu Ser Tyr Tyr Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg
                110                                     120
CCA AAT GCA CAA CGT TTC GGT ATC TCA AAC TAT TGC CAA ATT TAC CCA 432
Pro Asn Ala Gln Arg Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro
                                130
```

FIG. 1A

```
CCA AAT GCA AAC AAA ATT CGT GAA GCT TTG GCT CAA ACC CAC AGC GCT 480
Pro Asn Ala Asn Lys Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala
        140                                     150
ATT GCC GTC ATT ATT GGC ATC AAA GAT TTA GAC GCA TTC CGT CAT TAT 528
Ile Ala Val Ile Ile Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr
                        160
GAT GGC CGA ACA ATC ATT CAA CGC GAT AAT GGT TAC CAA CCA AAC TAT 576
Asp Gly Arg Thr Ile Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr
170                                     180
CAC GCT GTC AAC ATT GTT GGT TAC AGT AAC GCA CAA GGT GTC GAT TAT 624
His Ala Val Asn Ile Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr
                190                                     200
TGG ATC GTA CGA AAC AGT TGG GAT ACC AAT TGG GGT GAT AAT GGT TAC 672
Trp Ile Val Arg Asn Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr
                                210
GGT TAT TTT GCT GCC AAC ATC GAT TTG ATG ATG ATT GAA GAA TAT CCA 720
Gly Tyr Phe Ala Ala Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro
        220         222
TAT GTT GTC ATT CTC TAAACAAAAAGACAATTTCTTATATGATTGTCACTAATTTATT 778
Tyr Val Val Ile Leu
TAAAATCAAAATTTTAGAAAATGAATAAATTCATTCACAAAATTAAAAAAAAAAAAAAAAAA 841
AAAAAAAAAAAAAAAA 857
```

FIG. 1B

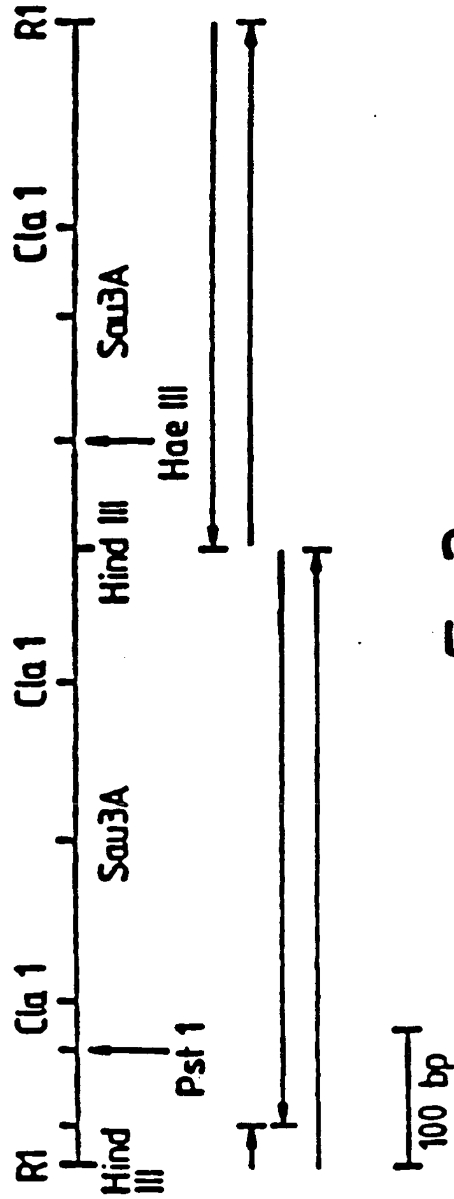

R1
Cla 1
Sau3A
Hae III
Hind III
Cla 1
Sau3A
Cla 1
Pst 1
R1
Hind III
100 bp

FIG.2.

```
            1                                       10
Der p 1   Thr Asn Ala Cys Ser Ile Asn  -  Gly Asn Ala Pro
Der f 1   Thr Ser Ala Cys Arg Ile Asn Ser Val Asn Val Pro

                                        20
Der p 1   Ala Glu Ile Asp Leu Arg Gln Met
Der f 1   Ser Glu Leu Asp Leu Arg Ser Leu
```

FIG.3.

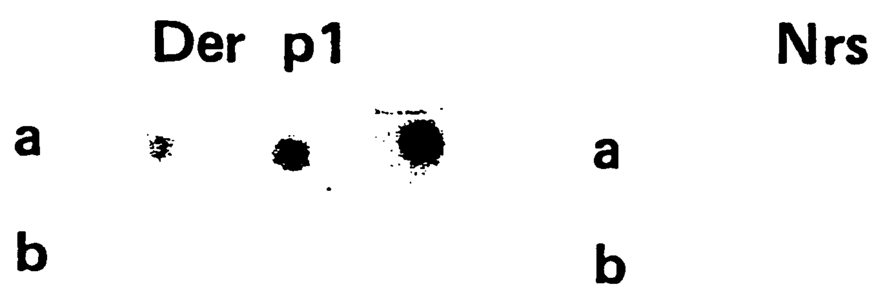



FIG. 4

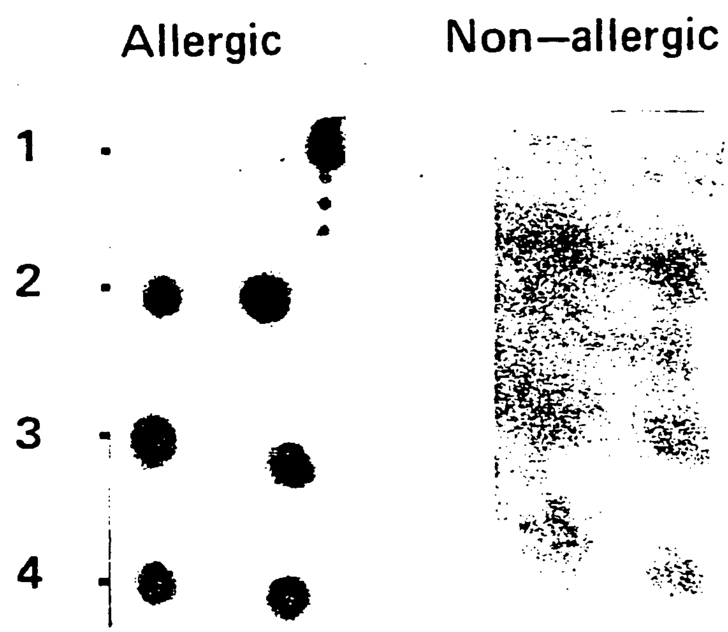



FIG. 5

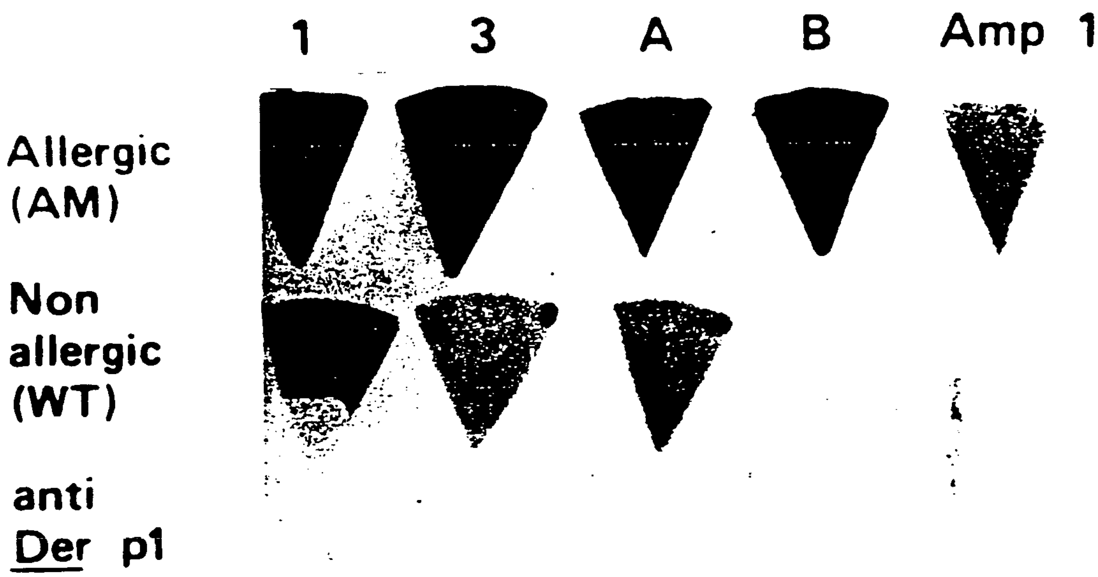



FIG.6

CACAAATTCTTCTTTCTTCCTTACTACTGATCATTAATCTGAAAACAAAACCAAACAAACCAT

-16 -10
TCAAAATGATG TAC AAA ATT TTG TGT CTT TCA TTG TTG GTC GCA GCC GTT
Met Tyr Lys Ile Leu Cys Leu Ser Leu Leu Val Ala Ala Val

-1 1 10
GCT CGT GAT CAA GTC GAT GTC AAA GAT TGT GCC AAT CAT GAA ATC AAA
Ala Arg Asp Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile Lys

20 30
AAA GTT TTG GTA CCA GGA TGC CAT GGT TCA GAA CCA TGT ATC ATT CAT
Lys Val Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile His

40
CGT GGT AAA CCA TTC CAA TTG GAA GCC GTT TTC GAA GCC AAC CAA AAC
Arg Gly Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln Asn

50 60
ACA AAA ACG GCT AAA ATT GAA ATC AAA GCC TCA ATC GAT GGT TTA GAA
Thr Lys Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu Glu

FIG. 7A

```
                                    70
GTT GAT GTT CCC GGT ATC GAT CCA AAT GCA TGC CAT TAC ATG AAA TGC
Val Asp Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys

     80                                         90
CCA TTG GTT AAA GGA CAA CAA TAT GAT ATT AAA TAT ACA TGG AAT GTT
Pro Leu Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val

                     100                                    110
CCG AAA ATT GCA CCA AAA TCT GAA AAT GTT GTC GTC ACT GTT AAA GTT
Pro Lys Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Val

                                         120
ATG GGT GAT GAT GGT GTT TTG GCC TGT GCT ATT GCT ACT CAT GCT AAA
Met Gly Asp Asp Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys

         129
ATC CGC GAT TAAATCAAACAAAATTTATTGATTTTGTAATCACAAATGATTGATTTTCTT
Ile Arg Asp

TCCAAAAAAAAAATAAATAAAATTTTGGGAATTC   581
```

FIG. 7B

```
Der p II   DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGKPF
                     •     • •     •

Der f II   DQVDVKD?ANNEIKKVMVDG?HGSDP?IIHRGKPF
```

• - non homologous residues.

FIG.8.

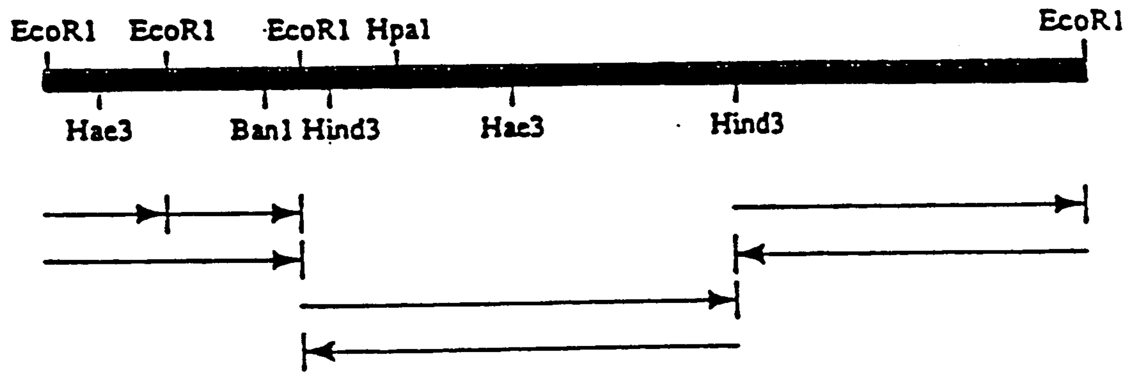



FIG. 9

```
                                              -98
GAATTCCGTTTTCTTCCATCAAAATTAAAAATTCATCAAAA ATG AAA TTC GTT TTG GCC ATT   62
                                          Met Lys Phe Val Leu Ala Ile
    -90                                    -80
GCC TCT TTG TTG GTA TTG AGC ACT GTT TAT GCT CGT CCA GCT TCA ATC AAA ACT  116
Ala Ser Leu Leu Val Leu Ser Thr Val Tyr Ala Arg Pro Ala Ser Ile Lys Thr
            -70                                     -60
TTT GAA GAA TTC AAA AAA GCC TTC AAC AAA AAC TAT GCC ACC GTT GAA GAG GAA  170
Phe Glu Glu Phe Lys Lys Ala Phe Asn Lys Asn Tyr Ala Thr Val Glu Glu Glu
                    -50                                     -40
GAA GTT GCC CGT AAA AAC TTT TTG GAA TCA TTG AAA TAT GTT GAA GCT AAC AAA  224
Glu Val Ala Arg Lys Asn Phe Leu Glu Ser Leu Lys Tyr Val Glu Ala Asn Lys
                            -30                                     -20
GGT GCC ATC AAC CAT TTG TCC GAT TTG TCA TTG GAT GAA TTC AAA AAC CGT TAT  278
Gly Ala Ile Asn His Leu Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Tyr
                                    -10
TTG ATG AGT GCT GAA GCT TTT GAA CAA CTC AAA ACT CAA TTC GAT TTG AAT GCC  332
Leu Met Ser Ala Glu Ala Phe Glu Gln Leu Lys Thr Gln Phe Asp Leu Asn Ala
-1   1                                  10
GAA ACA AGC GCT TGC CGT ATC AAT TCG GTT AAC GTT CCA TCG GAA TTG GAT TTA  386
Glu Thr Ser Ala Cys Arg Ile Asn Ser Val Asn Val Pro Ser Glu Leu Asp Leu
        20                                  30
CGA TCA CTG CGA ACT GTC ACT CCA ATC CGT ATG CAA GGA GGC TGT GGT TCA TGT  440
Arg Ser Leu Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly Cys Gly Ser Cys
                40                                  50
TGG GCT TTC TCT GGT GTT GCC GCA ACT GAA TCA GCT TAT TTG GCC TAC CGT AAC  494
Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala Tyr Leu Ala Tyr Arg Asn
```

FIG. 10A

```
                                      60                                                                       70
ACG TCT TTG GAT CTT TCT GAA CAG GAA CTC GTC GAT TGC GCA TCT CAA CAC GGA   548
Thr Ser Leu Asp Leu Ser Glu Gln Glu Leu Val Asp Cys Ala Ser Gln His Gly
                                    80
TGT CAC GGC GAT ACA ATA CCA AGA GGC ATC GAA TAC ATC CAA CAA AAT GGT GTC   602
Cys His Gly Asp Thr Ile Pro Arg Gly Ile Glu Tyr Ile Gln Gln Asn Gly Val
 90                                     100
GTT GAA GAA AGA AGC TAT CCA TAC GTT GCA CGA GAA CAA CGA TGC CGA CGA CCA   656
Val Glu Glu Arg Ser Tyr Pro Tyr Val Ala Arg Glu Gln Arg Cys Arg Arg Pro
        110                                     120
AAT TCG CAA CAT TAC GGT ATC TCA AAC TAC TGC CAA ATT TAT CCA CCA GAT GTG   710
Asn Ser Gln His Tyr Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asp Val
                130                                     140
AAA CAA ATC CGT GAA GCT TTG ACT CAA ACA CAC ACA GCT ATT GCC GTC ATT ATT   764
Lys Gln Ile Arg Glu Ala Leu Thr Gln Thr His Thr Ala Ile Ala Val Ile Ile
                        150                                     160
GGC ATC AAA GAT TTG AGA GCT TTC CAA CAT TAT GAT GGA CGA ACA ATC ATT CAA   818
Gly Ile Lys Asp Leu Arg Ala Phe Gln His Tyr Asp Gly Arg Thr Ile Ile Gln
                                170
CAT GAC AAT GGT TAT CAA CCA AAC TAT CAT GCC GTC AAC ATT GTC GGT TAC GGA   872
His Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile Val Gly Tyr Gly
180                                     190
AGT ACA CAA GGC GAC GAT TAT TGG ATC GTA CGA AAC AGT TGG GAT ACT ACC TGG   926
Ser Thr Gln Gly Asp Asp Tyr Trp Ile Val Arg Asn Ser Trp Asp Thr Thr Trp
        200                                     210
GGA GAT AGC GGA TAC GGA TAT TTC CAA GCC GGA AAC AAC CTC ATG ATG ATC GAA   980
Gly Asp Ser Gly Tyr Gly Tyr Phe Gln Ala Gly Asn Asn Leu Met Met Ile Glu
                220     223
CAA TAT CCA TAT GTT GTA ATC ATG TGAACATTTGAAATTGAATATATTTATTTGTTTTCAAAAT 1044
Gln Tyr Pro Tyr Val Val Ile Met
AAAAACAACTACTCTTGCGAGTATTTTTTACTCGGAATTC 1084
```

FIG. 10B

```
                   10        20        30        40        50        60
Der p 1    TNACSING*NAPAEIDLRQMRTVTPIRMQGGCGSCWAFSGVAATESAYLAHRNQSLDLAEQE
Der f 1    .S..R..SV.V.S.L...SL.........................Y..T....S...

                   70        80        90       100       110       120
Der p 1    LVDCASQHGCHGDTIPRGIEYIQHNGVVQESYYRYVAREQSCRRPNAQRFGISNYCQIYPPN
Der f 1    ......................Q....E.RS.P......R.....S.HY..........D

                  130       140       150       160       170       180
Der p 1    ANKIREALAQTHSAIAVIIGIKDLDAFRHYDGRTIIQRDNGYQPNYHAVNIVGYSNAQGVDY
Der f 1    VKQ.....T...T..........R..Q.........H................GST..D..

                  190       200       210       220
Der p 1    WIVRNSWDTNWGDNGYGYFAANIDLMMIEEYPYVVIL
Der f 1    .........T...S.....Q.GNN.....Q......M
```

FIG. 11

FIG. 12 A

```
Cathepsin H    MWTALPLLCAGAWLLSAGATA----------------------ELTY-NA-IEKFH---FTSWMKQHQKTY-SS-
Cathepsin L    MTPLLLLAVLCLGTALA---------------------TPKFDQ-TF-NAQWH-----QWKSTHRRLY-GT-
Papain         MAMIPSISKLLFVAICLFVYMGLSFG------------DFSIVGYSQNDLTS-TE-RLIQL---FESWMLKHNKIYKNI-
Aleurain       MAHARVLLLALAVLATAAVAYASSSSFADSNPIRPVTDRAASTLESAVLGALGRTRHALRFARFAVRYGKSYESA-
CP1            MKVILLFVLAVFTVF----------------VSSRGIPPEEQ-SQ-FLEFQ---DKFNKKYSHEEY-LE-
CP2            MRLLVFLILLIFVNFSFA-----------------NVRPNGRRFS-ES-QYRTA---FTEWTLKFNRQY-SS-
Cathepsin B    MWWSLIPLSCLLALTSA-----------------------------------------HDK---PS-
CTLA-2α        MVSICEQKLQHFSAVFLLILCLGMMSA----------------------------APPPDPSLDNEWKEWKTKFAKAYNLN-
CTLA-2β        MVSICEQKLQHFSAVFLLILCLGMMSA----------------------------APSPDPSLDNEWKEWKTTFAKAYSLD-
MCP            NLLLLAVLCLGTALA-----------------------------TPKFDQTFSAEWHQWKSTHRRLY-GT-
Der f I        MKFVLAIASLLVLSTVYA-------------------------------RPASIKTFEEFKKAFNKNYATVE
                                                                          **    . *
                                                                                  *
```

```
Cathepsin H    REYSHRLQVFANNWRKIQAHN--QRN--HTFKMG--LNQFSDMSFAEIKIKYL-WSE-PQNCS--AT-KS--NYL--RGTGP
Cathepsin L    NEEEWRRAVWEKNMRMIQIHNGEYSNGKHGFIHE--MNAFGDMTNEEFRQIVN-GYR-HQKHK--KG-RL--FQE--PLMLQ
Papain         DEKIYRFEIFKDNLKYIDETN--KKN--NSYWLG--LNVFADMSNDEFKEKYT-GSI-AGNYT--TTELSYEEVL-NDGDVN
Aleurain       AEVRRRFRIFSESLEEVRSTN--RKG--LPYRLG--INRFSDMSWEEFQATRL-GA--AQTCS--ATLAG--NHL-MRDAAA
CP1            RFEIFKSNLGKIEELNLIAIN--HKA--DT-KFG--VNKFADLSSDEFKNYYLNNKEAIFTDD--LP-VA--DYLDDEFINS
CP2            SEFSNRYSIFKSNMDYVDNWN-SKGD--SQTVLG--LNNFADITNEEYRKTYL-GTR-VNAHSYNGYDGR--EVLNVEDLQT
Cathepsin B    ---FHPLS---DDM--INYIN--KQN--TTWQAG--RN-EYNV-DISYLKKPC-GTV-LGGPK--LP-ER--VGF--SEDIN
CTLA-2α        NEERHRRLVWEENKKKIEAHNADYEQGKTSFYMG--LNQFSDLTPEEFKTNCY-GNSLNRGEM
CTLA-2β        DEERHRRLMWEENKKKIEAHNADYERGKTSFYMG--LNQFSDLTPEEFRTNCC-GSSMCRGEM
MCP            NEEEWRRAIWEKNMRMIQLHNGEYSNGQHGFSME--MNAFGDMTNEEFRQVVN-GYRHQKHKK
Der p I                                           --KNRFL-MS-AEAFEH-L-KTQFRLNAE
Actinidin                LRFIDEHNAD-TNR--SYKVG--LNQFADLTGEEFRSTYL-G
Der f I        EEEVARKN-FLESLKYVEA-NKGAINHLSDLSLDEFKNRYL-MS-AEAFEQ-L-KTQFDLNAE
               ** *     *      *   *     *  * * *..  **   * *
               *  *            *            * *     *
```

FIG. 12B

FIG. 13A

Der p1

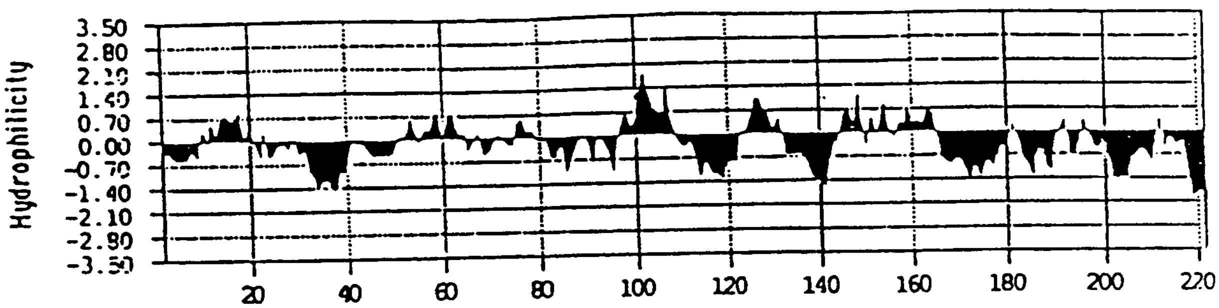
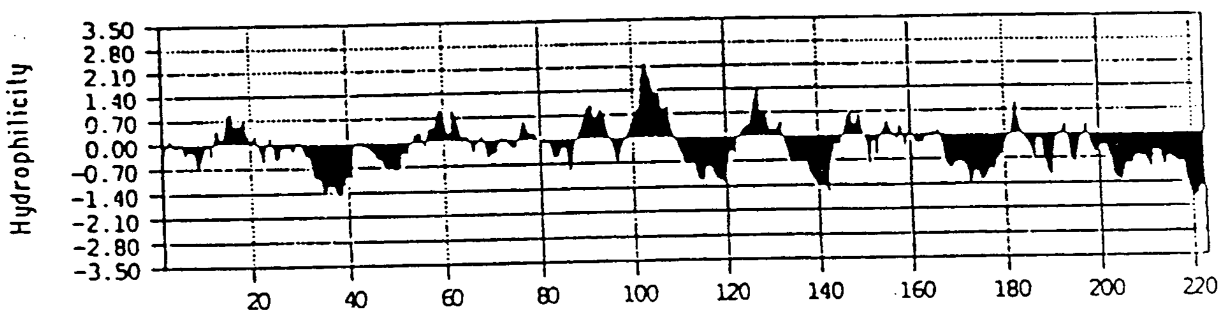



FIG. 13B

Der f1



```
                                       10
GAT CAA GTC GAT GTT AAA GAT TGT GCC AAC AAT GAA ATC AAA AAA GTA ATG     51
Asp Gln Val Asp Val Lys Asp Cys Ala Asn Asn Glu Ile Lys Lys Val Met

        20                                      30
GTC GAT GGT TGC CAT GGT TCT GAT CCA TGC ATC ATC CAT CGT GGT AAA CCA    102
Val Asp Gly Cys His Gly Ser Asp Pro Cys Ile Ile His Arg Gly Lys Pro

                    40                                      50
TTC ACT TTG GAA GCC TTA TTC GAT GCC AAC CAA AAC ACT AAA ACC GCT AAA    153
Phe Thr Leu Glu Ala Leu Phe Asp Ala Asn Gln Asn Thr Lys Thr Ala Lys

                                60
ACT GAA ATC AAA GCC AGC CTC GAT GGT CTT GAA ATT GAT GTT CCC GGT ATT    204
Thr Glu Ile Lys Ala Ser Leu Asp Gly Leu Glu Ile Asp Val Pro Gly Ile

    70                                      80
GAT ACC AAT GCT TGC CAT TTT ATG AAA TGT CCA TTG GTT AAA GGT CAA CAA    255
Asp Thr Asn Ala Cys His Phe Met Lys Cys Pro Leu Val Lys Gly Gln Gln

                    90                                      100
TAT GAT GCC AAA TAT ACA TGG AAT GTG CCG AAA ATT GCA CCA AAA TCT GAA    306
Tyr Asp Ala Lys Tyr Thr Trp Asn Val Pro Lys Ile Ala Pro Lys Ser Glu

                            110
AAC GTT GTC GTT ACA GTC AAA CTT GTT GGT GAT AAT GGT GTT TTG GCT TGC    357
Asn Val Val Val Thr Val Lys Leu Val Gly Asp Asn Gly Val Leu Ala Cys

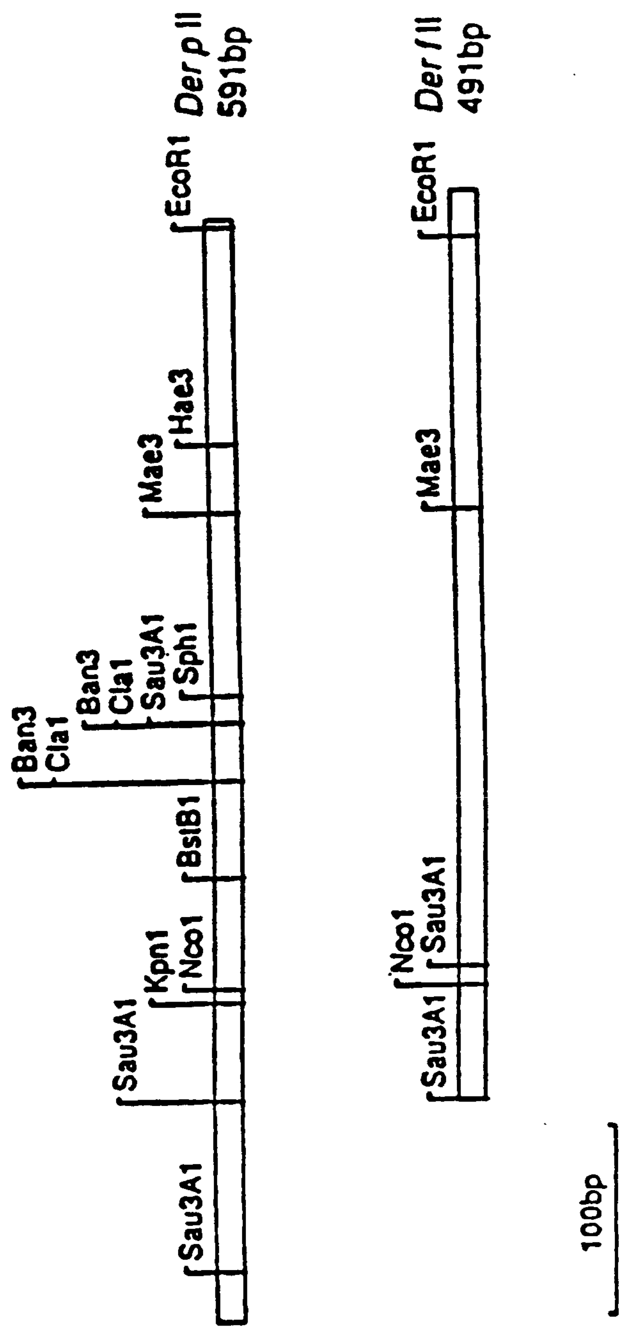
120                                         129
GCT ATT GCT ACC CAC GCT AAA ATC CGT GAT TAAAAAAAAAAAATAAATATGAAAATT    414
Ala Ile Ala Thr His Ala Lys Ile Arg Asp

TTCACCAACATCGAACAAAATTCAATAACCAAAATTTGAATCAAAAACGGAATTCCAAGCTGAGCGC    481

CGGTCGCTAC                                                             491
```

FIG. 14

FIG. 15


Der p II
591bp
EcoR1
Hae3
Mae3
Sph1
Sau3A1
Cla1
Ban3
Cla1
Ban3
BslB1
Nco1
Kpn1
Sau3A1
Sau3A1
Der f II
491bp
EcoR1
Mae3
Sau3A1
Nco1
Sau3A1
100bp

```
Dp II:  CACAAATTCTTCTTTCTTCCTTACTACTGATCATTAATCTGAAAACAAAACCAAACAAACCAT   63

                 -16                       -10
Dp II:  TCAAAATGATG TAC AAA ATT TTG TGT CTT TCA TTG TTG GTC GCA GCC GTT   113
                Met Tyr Lys Ile Leu Cys Leu Ser Leu Leu Val Ala Ala Val

            -1  1
Dp II:  GCT CGT GAT CAA GTC GAT GTC AAA GAT TGT GCC AAT CAT GAA ATC AAA   161
        Ala Arg Asp Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile Lys

Df II:          ... ... ... ... ..T ... ... ... ... ..C A.. ... ... ...   42
                                                        Asn

                            20                                     30
Dp II:  AAA GTT TTG GTA CCA GGA TGC CAT GGT TCA GAA CCA TGT ATC ATT CAT   209
        Lys Val Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile His

Df II:  ... ..A A.. ..C GAT ..T ... ... ... ..T ..T ... ..C ... ..C ...   90
            Met     Asp                     Asp

                                             40
Dp II:  CGT GGT AAA CCA TTC CAA TTG GAA GCC GTT TTC GAA GCC AAC CAA AAC   257
        Arg Gly Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln Asn

Df II:  ... ... ... ... ... ACT ... ... ... T.A ... ..T ... ... ... ...   138
                            Thr             Leu     Asp
```

FIG. 16A

```
                    50                                      60
Dp II:  ACA AAA ACG GCT AAA ATT GAA ATC AAA GCC TCA ATC GAT GGT TTA GAA   305
        Thr Lys Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu Glu

Df II:  ..T ... ..C ... ... .C. ... ... ... ... AGC C.. ... ... C.T ...   186
                            Thr                     Leu

                                    70
Dp II:  GTT GAT GTT CCC GGT ATC GAT CCA AAT GCA TGC CAT TAC ATG AAA TGC   353
        Val Asp Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys

Df II:  A.. ... ... ... ... ..T ... A.C ... ..T ... ... .TT ... ... ..T   234
        Ile                         Thr                 Phe

            80                                      90
Dp II:  CCA TTG GTT AAA GGA CAA CAA TAT GAT ATT AAA TAT ACA TGG AAT GTT   401
        Pro Leu Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val

Df II:  ... ... ... ... ..T ... ... ... ... GCC ... ... ... ... ... ..G   282
                                             Ala

                            100                                     110
Dp II:  CCG AAA ATT GCA CCA AAA TCT GAA AAT GTT GTC GTC ACT GTT AAA GTT   449
        Pro Lys Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Val

Df II:  ... ... ... ... ... ... ... ... ..C ... ... ..T ..A ..C ... C..   330
                                                                    Leu
```

FIG. 16B

FIG. 16C

```
                                                      120
Dp II:  ATG GGT GAT GAT GGT GTT TTG GCC TGT GCT ATT GCT ACT CAT GCT AAA   497
        Met Gly Asp Asp Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys

Df II:  G.T ... ... A.. ... ... ... ..T ..  ... ... ... ..C ..C ... ...   378
        Val         Asn

                  129
Dp II:  ATC CGC GAT TAA ATCAAACAAAATTTATTGATTTTGTAATCACAAATGATTGATTTTCTT  557
        Ile Arg Asp END

Df II:  ... ..T ... ... .AA...A...TAAATA...AAA.T.TCA.CA.C.CGAAC.AAA.TCA  438

Dp II:  TCCAAAAAAAAAATAAATAAAATTTTGGGAATTC                                591

Df II:  ATA.CC....TTTG..TC....AC___GGAATTC                                469
```

FIG. 17A

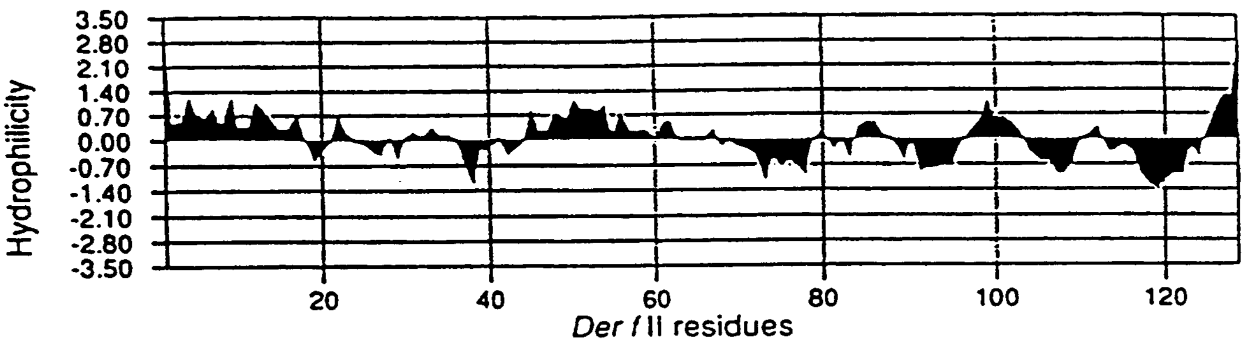
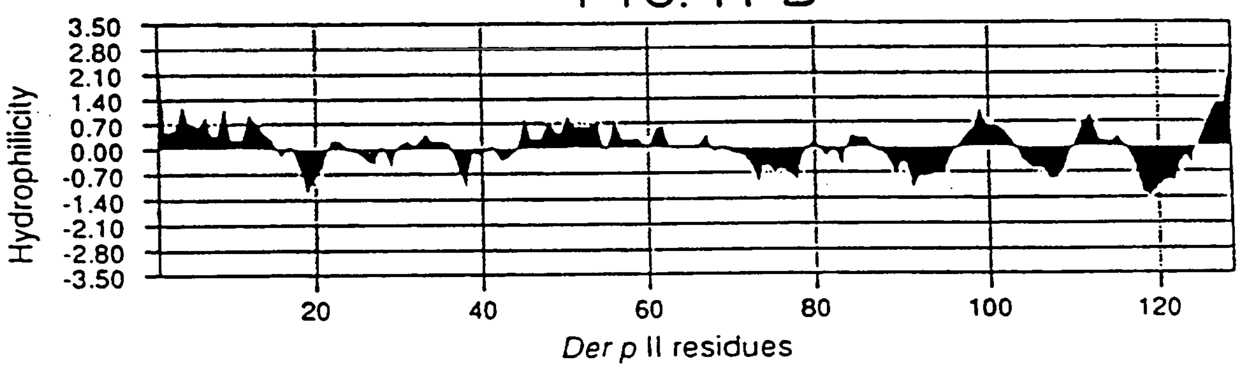



FIG. 17B



```
                  10          20          30          40          50          60
Der p I (a)  TNACSINGNA  PAEIDLRQMR  TVTPIRMQGG  CGSCWAFSGV  AATESAYLAH  RNQSLDLAEQ
Der P I (b)  ----------  ----------  ----------  ----------  ----------  ----------
Der p I (c)  ----------  ----------  ----------  ----------  ---------Y  ----------
Der p I (d)  ----------  ----------  ----------  ----------  ---------Y  ----------
                                                            ----------  ----------

                  70          80          90         100         110         120
Der p I (a)  ELVDCASQHG  CHGDTIPRGI  EYIQHNGVVQ  ESYYRYVARE  QSCRRPNAQR  FGISNYCQIY
Der P I (b)  ----------  ----------  ----------  ----------  ----------  ----------
Der p I (c)  ----------  ----------  K---------  ----------  ----------  ----------
Der p I (d)  ----------  ----------  ----------  ----------  ----------  ----------
                                                            ----------  ----------

                 130         140         150         160         170         180
Der p I (a)  PPNANKIREA  LAQTHSAIAV  IIGIKDLDAF  RHYDGRTIIQ  RDNGYQPNYH  AVNIVGYSNA
Der P I (b)  ---V------  ----------  ----------  ----------  ----------  ----------
Der p I (c)  ---V------  ----------  ----------  ----------  ----------  ----------
Der p I (d)  ----------  -----T----  ----------  ----------  ----------  ----------
Der p I (e)                                      ----------  ----------  ----------

                 190         200         210         220
Der p I (a)  QGVDYWIVRN  SWDTNWGDNG  YGYFAANIDL  MMIEEYPYVV  IL
Der P I (b)  ----------  ----------  ----------  ----------  --
Der p I (c)  ----------  ----------  ----------  ----Q-----  --
Der p I (d)  ----------  ----------  ----------  ----------  --
Der p I (e)  ----------  ----------  ----------  ----------  --
```

FIG. 18

```
                          10        20        30        40        50
Der p II (c)     DQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGKPFQLEAVFEANQNTKTAK
         (1)     ..........H.....L.P.....E..........Q...V.E....T....
         (2)     ..........H.....L.P.....E..........Q...V.E....S....
Der f II         ..........N.....M.D.....D..........T...L.D....T....

                          60        70        80        90       100
Der p II (c)     IEIKASIDGLEVDVPGIDPNACHYMKCPLVKGQQYDIKYTWNVPKIAPKSE
         (1)     ......I...........P....YM...........I..............
         (2)     ......I...........P....YM...........I..............
Der f II         ......L...........T....FM...........A..............
                                         V           I

                         110       120
Der p II (c)     NVVVTVKVMGDDGVLACAIATHAKIRD
         (1)     .......VM.DD......A....I..
         (2)     .......VM.ND......A....L..
Der f II         .......LV.DN......A....I..
                        I          G
```

FIG. 19

```
                        10        20        30        40        50        60
pFL1            DQVDVKDCANNEIKKVMVPGCHGSEPCIIHRGKPFTLEALFDANQNTKTAKIEIKASLDGLE
pFL2            ..........N.........................................I.I.......
MT 3            ..........N.........................................I.T.......
MT 5  (1-92)    ..........S.........................................I.I.......
MT18  (1-84)    ..........N.........................................I.I.......
MT16  (1-70)    ..........N.........................................T.I.......

                      70        80        90       100       110       120       130
pFL1            IDVPGIDTNACHFVKCPLVKGQQYDIKYTWNVPKIAPKSENVVVTVKLIGDNGVLACAIATHAKIRD
pFL2            .............M..........A.....................V....................
MT 3            .............M..........A.....................V....................
MT 5            .............M..........I....
MT18            .............M........
```

FIG. 20

```
GAATTCCTTT TTTTTTCTTT CTCTCTCTAA AATCTAAAAT CCATCCAAC ATG AAA ATT    58
                                                      Met Lys Ile
                                                      -98

GTT TTG GCC ATC GCC TCA TTG TTG GCA TTG AGC GCT GTT TAT GCT CGT    106
Thr Leu Ala Ile Ala Ser Leu Leu Ala Leu Ser Ala Val Tyr Ala Arg
-95                 -90                 -85                 -80

CCA TCA TCG ATC AAA ACT TTT GAA GAA TAC AAA AAA GCC TTC AAC AAA    154
Pro Ser Ser Ile Lys Thr Phe Glu Glu Tyr Lys Lys Ala Phe Asn Lys
                -75                 -70                 -65

AGT TAT GCT ACC TTC GAA GAT CAA GAA GCT GCC CGT AAA AAC TTT TTG    202
Ser Tyr Ala Thr Phe Glu Asp Glu Glu Ala Ala Arg Lys Asn Phe Leu
            -60                 -55                 -50

GAA TCA GTA AAA TAT GTT CAA TCA AAT GGA GGT GCC ATC AAC CAT TTG    250
Glu Ser Val Lys Tyr Val Gln Ser Asn Gly Gly Ala Ile Asn His Leu
        -45                 -40                 -35

TCC GAT TTG TCG TTG GAT GAA TTC AAA AAC CGA TTT TTG ATG AGT GCA    298
Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Phe Leu Met Ser Ala
    -30                 -25                 -20

GAA GCT TTT GAA CAC CTC AAA ACT CAA TTC GAT TTG AAT GCT GAA ACT    346
Glu Ala Phe Glu His Leu Lys Thr Gln Phe Asp Leu Asn Ala Glu Thr
-15                 -10                 -5                  -1   1

AAC GCC TGC AGT ATC AAT GGA AAT GCT CCA GCT GAA ATC GAT TTG CGA    394
Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile Asp Leu Arg
            5                   10                  15
```

FIG. 21A

```
CAA ATG CGA ACT GTC ACT CCC ATT CGT ATG CAA GGA GGC TGT GGT TCA   442
Gln Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly Cys Gly Ser
         20                  25                  30

TGT TGG GCT TTC TCT GGT GTT GCC GCA ACT GAA TCA GCT TAT TTG GCT   490
Cys Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala Tyr Leu Ala
     35                  40                  45

CAC CGT AAT CAA TCA TTG GAT CTT GCT GAA CAA GAA TTA GTC GAT TGT   538
His Arg Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu Val Asp Cys
 50                  55                  60                  65

GCT TCC CAA CAC GGT TGT CAT GGT GAT ACC ATT CCA CGT GGT ATT GAA   586
Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg Gly Ile Glu
                 70                  75                  80

TAC ATC CAA CAT AAT GGT GTC GTC CAA GAA AGC TAC TAT CGA TAC GTT   634
Tyr Ile Gln His Asn Gly Val Val Gln Glu Ser Tyr Tyr Arg Tyr Val
             85                  90                  95

GCA CGA GAA CAA TCA TGC CGA CGA CCA AAT GCA CAA CGT TTC GGT ATC   682
Ala Arg Glu Gln Ser Cys Arg Arg Pro Asn Ala Gln Arg Phe Gly Ile
        100                 105                 110

TCA AAC TAT TGC CAA ATT TAC CCA CCA AAT GCA AAC AAA ATT CGT GAA   730
Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn Ala Asn Lys Ile Arg Glu
    115                 120                 125
```

FIG. 21B

```
GCT TTG GCT CAA ACC CAC AGC GCT ATT GCC GTC ATT ATT GGC ATC AAA      778
Ala Leu Ala Gln Thr His Ser Ala Ile Ala Val Ile Ile Gly Ile Lys
130                 135                 140                 145

GAT TTA GAC GCA TTC CGT CAT TAT GAT GGC CGA ACA ATC ATT CAA CGC      826
Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile Ile Gln Arg
                150                 155                 160

GAT AAT GGT TAC CAA CCA AAC TAT CAC GCT GTC AAC ATT GTT GGT TAC      874
Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala Val Asn Ile Val Gly Tyr
            165                 170                 175

AGT AAC GCA CAA GGT GTC GAT TAT TGG ATC GTA CGA AAC AGT TGG GAT      922
Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn Ser Trp Asp
        180                 185                 190

ACC AAT TGG GGT GAT AAT GGT TAC GGT TAT TTT GCT GCC AAC ATC GAT      970
Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr Phe Ala Ala Asn Ile Asp
    195                 200                 205

TTG ATG ATG ATT GAA GAA TAT CCA TAT GTT GTC ATT CTC TAAACAAAAA      1019
Leu Met Met Ile Glu Glu Tyr Pro Tyr Val Val Ile Leu
210                 215                 220

GACAATTTCT TATATGATTG TCACTAATTT ATTTAAAATC AAAATTTTTA GAAAATGAAT  1079

AAATTCATTC ACAAAAATTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA  1139

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA                               1172
```

FIG. 21C

# INTERNATIONAL SEARCH REPORT

International Application No
US 93/08518

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 5 C12N15/12 C12P21/02 C07K15/08 A61K39/35

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO,A,92 04445 (THE WESTERN AUSTRALIAN RESEARCH INSTITUTE FOR CHILD HEALTH LTD.) 19 March 1992 see figure 9 --- | 1-6 |
| X | WO,A,88 10297 (PRINCESS MARGARET CHILDREN'S MEDICAL RESEARCH FOUNDATION (INC.) ET AL.) 29 December 1988 --- | 1,3,4 |
| X | EP,A,0 445 971 (ASAHI BREWERIES, LTD.) 11 September 1991 see page 3, line 35 - page 7 ----- | 2,5,6 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 24 January 1994 | 22-02-1994 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Cupido, M |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/08518

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : although claims 4 and 6 are directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

information on patent family members

International Application No
PCT/US 93/08518

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| WO-A-9204445 | 19-03-92 | AU-A- | 8506291 | 30-03-92 |
| | | CA-A- | 2091449 | 12-03-92 |
| | | EP-A- | 0548200 | 30-06-93 |
| WO-A-8810297 | 29-12-88 | AU-A- | 1959888 | 19-01-89 |
| | | EP-A- | 0362290 | 11-04-90 |
| | | JP-T- | 3501920 | 09-05-91 |
| EP-A-0445971 | 11-09-91 | JP-A- | 3254683 | 13-11-91 |
| | | AU-B- | 640450 | 26-08-93 |
| | | AU-A- | 7127791 | 05-09-91 |